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THE RELATION OF PHOSPHORUS DEFICIENCY TO THE UTILIZATION OF FEED IN DAIRY CATTLE

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SUMMARY

The results of an investigation, studying the problem of lowered feed utilization in phosphorus-deficient cattle, are reported. Data also are presented on the balance of nitrogen, calcium, and phosphorus in deficient, animals.

The observation of previous workers, that a shortage of phosphorus in the ration becomes a limiting factor in the economical utilization of feeds, is substantiated.

The problem of feed utilization has been investigated from three possible angles: First, the relation of phosphorus deficiency to digestion; second, the determination of possible abnormal losses of energy in the visible excreta of affected animals; and third, the relation of phosphorus intake to the energy metabolism of dairy cattle.

The results obtained would indicate that: (1) Phosphorus deficiency does not depress the digestive functions of the animal. Lactating dairy cows in a condition of aphosphorosis were found to digest their feed as completely as the normal control. (2) By means of gross energy determinations of the feed and visible excreta no abnormal losses of energy were demonstrated in the excreta of phosphorus-deficient animals. (3) Oxygen-consumption measurements made by means of a portable metabolism apparatus indicated a higher energy metabolism for animals in the phosphorus-deficient condition.

A marked symptom of the low-phosphorus condition was the failure of appetite observed in all experimental animals on the deficient ration. This failure of appetite alone, however, would not explain the losses in weight noted in all animals, since these losses began while the cows were consuming sufficient nutrients to allow for appreciable gains.

The addition of the phosphorus supplement to the deficient ration produced an immediate response of the appetite in most cases. More economical utilization of the nutrients consumed also resulted from the addition of the supplement, as indicated by the gains in weight of the experimental animals.

In using dairy cows of good producing ability it was found possible to induce symptoms of aphosphorosis rather rapidly on the deficient ration. A shortage of phosphorus in the ration, however, did not prove so important a limiting factor in milk production as it did in body maintenance.

As noted by earlier investigators, the marked effect of a shortage of phosphorus on the inhibition of œstrum was observed in the present experiment.

In preliminary experiments with rabbits it was not found possible to induce symptoms of aphosphorosis at all comparable to those observed in dairy cattle.

It is interesting to note that, in seeking suitable low-phosphorus roughage for use in the present investigation, in certain sections of Kansas, prairie hay was found having as low phosphorus content as that reported for roughages from other areas where this deficiency disease is prevalent. Later investigation has shown that cattle in these areas exhibit typical symptoms of the disorder and respond readily to the feeding of phosphorus-containing supplements.

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THE RELATION OF PHOSPHORUS DEFICIENCY TO THE UTILIZATION OF FEED IN DAIRY CATTLE[1]

W. H. RIDDELL,[2] J. S. HUGHES, AND J. B. FITCH

STATEMENT OF PROBLEM

Mineral deficiencies in the rations of cattle occasion large economic losses to the live-stock industry throughout the world. While such problems had been recognized as long ago as the middle of the past century, it is only within the last twenty years that serious investigation has been directed to the primary causes. The reason for this, undoubtedly, lies in the fact that our knowledge of the importance of minerals in live-stock feeding is of comparatively recent origin. With a growing appreciation of the function of the mineral elements in the ration, the relation of mineral deficiencies to live-stock production became emphasized.

A lack of phosphorus in the forage has been demonstrated as one of the most common types of mineral deficiency affecting the live-stock industry. This condition has been found to result from a deficient supply of available phosphorus in the soil. Phosphorus-deficient areas now have been reported from almost every part of the world. In the United States surveys have shown the problem to be one of major importance in Minnesota, Montana, and the coastal plain of Texas. In Kansas, where the present investigation has been carried on, symptoms of phosphorus deficiency have been recognized in cattle maintained on upland soil in some of the southeastern counties. With increasing evidence of the widespread nature of this problem, it is likely that few states will escape some manifestation of this disorder.

A frequent observation in cases of aphosphorosis in cattle is the general lack of thrift and the poor condition of animals affected. This may accompany even reasonably good feeding practices. Where the deficiency is of long standing, anorexia, stunted growth, and little tendency to lay on flesh are commonly observed manifestations. It has been definitely established that there is a lowered efficiency in feed utilization. The phosphorus-deficient animal simply does not realize so much per unit of feed consumed. Eckles *(17)* in discussing this problem asks, "What is the explanation of the striking difference in the utilization of feed when the phosphorus is increased in a ration deficient in this element?" In seeking an answer to this interesting phenomenon the following questions may be raised more specifically:

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1. Contribution No. 86 from the Department of Dairy Husbandry and No. 172 from the Department of Chemistry.

2. The material in this bulletin was presented by W. H. Riddell in partial fulfillment of the requirements for the degree of doctor of philosophy, University of Minnesota, 1932. He is indebted to the late Dr. C. H. Eckles and to Dr. L. S. Palmer for suggesting the problem and for their helpful criticisms and suggestions in planning and carrying out the investigation.

1. Does the condition of aphosphorosis lower the digestibility of the ration and prevent normal utilization of the nutrients?

2. Does the condition resulting from phosphorus deficiency interfere with metabolism to the extent that the assimilated nutrients are not completely oxidized? It is well known that phosphorus plays an important role in the oxidation of carbohydrates in the body. Theiler *(40)* has suggested that "phosphorus plays a specific role in cellular metabolism; has what might be called a vitamin effect, which facilitates utilization of carbohydrate and protein in general tissue anabolism."

3. Another possibility is that phosphorus deficiency affects the metabolism of the animal organism in such a manner as to change its rate of basal metabolism. Such a condition of increased basal metabolism is found in certain types of goiter, which some investigators believe results from a deficiency of iodine in the diet.

It was with the hope of throwing further light on some of these problems that the present investigation was undertaken.

REVIEW OF LITERATURE.

UTILIZATION OF FEED IN PHOSPHORUS DEFICIENCY

No attempt will be made to review the very extensive literature. on the subject of phosphorus deficiency in live stock. This has been made available in two excellent reviews, one by Eckles, Becker, and Palmer *(16)*, and another more recently by Theiler and Green *(58)*.

Theiler and associates have made a very comprehensive study of the relation of phosphorus to the live-stock industry. Their investigations have been reported in a series of papers, the first of which appeared in 1920 *(39)*. In 1924 Theiler, Green, and Du Toit *(40)*, in their paper "Phosphorus in the Live Stock Industry," definitely established the significance of a low phosphorus supply on the growth and production of animals grazing on the veld in South Africa. In 1927 these workers *(41)* reported the experimental production of aphosphorosis in cattle with all the clinically recognizable symptoms of the naturally occurring disorder.

In a series of investigations started in 1923, and practically paralleling the work of the South African group, Eckles, Palmer, and associates *(16, 17, 54)* in this country had determined experimentally that a deficiency of phosphorus was the factor involved in the nutritional disorder which they were investigating in Minnesota. These workers concluded that "under extreme conditions, as in the area studied, a shortage of phosphorus becomes a limiting factor in the economic utilization of feeds and in the growth of cattle."

Theiler and associates *(40)* concluded that phosphorus was a limiting factor in growth and voluntary food consumption and a dominating factor in the maintenance of live weight under ordinary conditions of veld grazing. A marked increase in the rate of growth and the ability to lay on flesh was observed in South African range cattle on the addition of bone meal to phosphorus-deficient rations. Apparently the condition of the animal could be controlled at will through the manipulation of the phosphorus content of the ration fed. Records of ostephagia, live weight, and feed consumption were reported by these workers on large groups of animals, and the results with bone meal feeding were particularly striking. The cattle receiving bone meal ate more hay, increased more in weight—in one typical experiment, practically four times as much as the controls receiving no bone meal. Furthermore, it was noted that although more hay was consumed by the bone-meal-supplemented animals during the periods of more rapid increase, the difference was not sufficient to account for the extra body weight put on. As a result of these observations, Theiler concluded that "phosphorus-fed animals utilize their food better and put on more body weight per unit of food consumed."

Du Toit and Bisschop *(14)* have continued the South African investigations to include studies on a large scale with improved breeds of cattle and the possibilities of grading up the native cattle under conditions of veld grazing, in which the earlier findings have been confirmed and extended. More recently Du Toit and associates *(15)* have reported that "sheep receiving a ration deficient in phosphorus have shown no gain in weight, lost appetite, and shown a very low figure for the inorganic phosphorus of the blood."

In a more rigidly controlled study of the energy requirements per pound of gain for dairy cows on a low phosphorus ration, Eckles and Gullickson *(17)* reported that a plane of feeding of at least 1.20 per cent of that specified by the feeding standards in general use was necessary to even maintain the live weight of experimental animals. On the low-phosphorus basal ration the digestible nutrients required per pound of gain ranged between 6 and 15 pounds, whereas with a phosphorus supplement added to the ration this requirement dropped to one-fifth or a range of one to three pounds per pound of gain.

Similar results confirming the marked improvement in the general thrift and condition of affected animals, through the simple addition of bone meal or other phosphorus-containing supplement, have been reported frequently in the literature from this country and elsewhere.

EFFECT OF MINERAL DEFICIENCY ON DIGESTION

Forbes and Keith *(23)* have reviewed the work on the influence of phosphates on digestion. They conclude that "phosphates stand in such a significant relation to the digestion and utilization of food that their excretion into the alimentary tract must be regarded as of vital consequence." The possible significance of phosphates in relation to enzymatic processes in the animal body is also indicated.

The more specific evidence bearing on the effect of a lowered mineral intake on digestibility is limited. In October, 1929, after the present investigation had been started, Evans *(90)* reported the results of a series of digestion trials with swine suffering from prolonged calcium deficiency. Twenty-two trials showed no enhanced effect on the digestibility of the organic constituents of the food on adding calcium carbonate to a lime-deficient ration. One year later Woodman and Evans *(43)* reported the results of a digestion study with sheep, from which it was concluded that a deficiency of calcium and phosphorus in the forage did not cause it to be digested any less efficiently.

It would seem probable that the processes of digestion are more or less constant and little affected by dietary deficiencies of a mineral character. This would suggest that the prolonged feeding of a low-phosphorus ration in all probability would not materially affect digestion. However, since there are no published data on the coefficient of digestibility of animals in the advanced stages of aphosphorosis, the present investigation accordingly was planned, in one of its phases, to furnish definite information on this subject.

ENERGY LOSSES IN METABOLISM

It is the general belief that phosphorus plays an important role in the early stages of carbohydrate and fat metabolism. Shaffer *(35)* has stated that "if the view be correct that glucose oxidation takes place *via* lactic acid, phosphoric acid is equally concerned with carbohydrate metabolism."

It has been known for some time that phosphorus compounds have a special role in muscular contraction. The discovery of "phosphagen," a labile form of organic phosphate, in 1926, by Eggleton and Eggleton *(18)*, has changed our outlook on the energy exchanges in muscle. Hill *(27)*, in a recent review, has discussed the newer viewpoint concerning lactic acid and phosphagen formation in muscle.

Fiske *(21)* was one of the first to observe the decreased output of urinary phosphates after sugar ingestion, and much evidence has been advanced since, demonstrating changes in the mobile phosphate stores of the body during active carbohydrate assimilation. It is quite generally believed that glucose unites with phosphoric acid to form a more easily oxidizable compound, hexose-phosphate. Furthermore, lending additional emphasis to the importance of the part played by phosphorus in body oxidations are the *"in vitro"* experiments of recent years demonstrating the marked influence of phosphorus on sugar oxidating *(2, 19, 36, 42)*.

Since phosphorus is thought to play such an important part in body oxidations, it would not be surprising, in a condition where this constituent in the inorganic form frequently falls to one-fourth of the value found in normal blood, that the oxidation processes would be so altered as to result in the excretion of partially oxidized products. This condition, obviously, would cause loss of energy to the animal body. Such a loss is known to occur in diabetes, in which the acetone bodies resulting from incomplete oxidation of fatty acids are excreted. Also, in this connection, it is interesting to note that, in certain types of vitamin deficency, an increased output of carbon has been observed in the urine of dogs and rats

(6, 28). This increase was attributed to the elimination of partially oxidized carbon compounds. Collazo and Munilla *(11)* have also reported finding a large increase of either soluble substances in the urine of dogs on a poly-deficient diet.

In view of these considerations, and since there were no available data on energy losses in the excreta of animals on a phosphorus-deficient diet, it was thought advisable to determine whether any such losses occurred. Gross energy determinations on the urine of these animals were of particular interest, since the urine is especially the vehicle for elimination of metabolic products from the body.

ENERGY METABOLISM

There is an extensive literature relating to the energy metabolism of man and other species under normal conditions. The research in the field of basal metabolism, as expressed under abnormal conditions, has been adequately reviewed by Du Bois *(13)*.

The basal metabolism of cattle is obtained with difficulty, because of the uncertainty in securing a true fasting condition, due to the complexity of the digestive tract. As a result many of the data on energy changes in this species have been secured in the form of metabolism measurements under "standard" conditions other than fasting. An early review of the literature in this field was made by Armsby *(1)*, but the most recent and extensive compilation has been made by Brody *(8)*.

Considerable investigation has been done on the energy metabolism in cattle on different planes of nutrition, as reported by Benedict and Ritzman *(4)*; Forbes, Braman, Kriss, *et al. (24, 66);* Brody and coworkers *(8, 9, 10)*; Mitchell *et al. (32)*; and others. In general, it has been, observed that the heat production resulting from the consumption of a definite amount of nutrients is less at the lower planes of nutrition than at the higher levels.

Benedict and Ritzman *(4)* have conducted very elaborate studies on the effect of undernutrition on steers, and it was observed by these workers that "undernutrition in every instance had a very pronounced influence upon the metabolism in that after a few weeks of adjustment the total metabolism was greatly lowered."

It is a well known fact among investigators in this field that the rate of metabolism is changed in certain pathological conditions, such as exophthalmic goiter and other glandular disorders. In a few conditions, such as that mentioned, a marked increase in the metabolic rate has been observed, while in other cases, and more frequently, the opposite condition has been found to obtain. There is no evidence available, however, that any energy metabolism investigations have been conducted with cattle under conditions of disease or specific dietary deficiency.

In the vitamin field Drummond and Marrian *(12)* made a prolonged study of the gaseous metabolism of rats in various states of nutrition. Their results during progressive vitamin B deficiency showed that the oxygen consumption remains normal until the final stages of the disturbance.

It has been sufficiently well established that cattle in progressive stages of phosphorus deficiency on a liberal ration realize less per unit of feed consumed than the normal animal. Since, as Theiler *(40)* has stated, they are simply combusting the extra ration for no useful purpose, it would seem logical to assume that this may be due to an increase in their basal metabolism. As there are no data available on the energy metabolism of experimental animals in advanced stages of aphosphorosis, it was planned to secure information on this important question.

After the foregoing review of the literature, it was decided to divide the general plan of the investigation into three phases:

1. Determination of coefficient of digestibility.
2. Determination of metabolizable energy.
3. Study of the energy metabolism of dairy cows in a condition of aphosphorosis.

EXPERIMENTAL DATA

PRELIMINARY WORK WITH RABBITS

In an investigation of this kind laboratory experiments with small animals are often suggestive of methods for attacking the main problem. Starting in the fall of 1927 and continuing for 18 months, preliminary experiments with rabbits were carried on by one of the authors in the nutrition laboratory of the Biochemistry Division, University of Minnesota.

In this phase of the experiment 18 rabbits were used. The low-phosphorus ration fed, at first, consisted of prairie hay, coarsely ground oats, and cabbage. This was later modified considerably because of limited consumption by the rabbits. In the later stages of the experiment a typical basal ration consisted of tapioca, 80 per cent; egg albumin, 10 per cent; ground oats, 10 per cent; salt and calcium carbonate, 1 per cent each, fed with prairie hay and cabbage. These ingredients making up the concentrate ration, after thorough mixing were soaked with water and dextrinized. This ration had a phosphorus content of less than 0.1 per cent. In spite of frequent changes in the kind and proportion of the ingredients it was not found possible to prepare a ration that was readily consumed by the experimental animals.

While there was abundant evidence of undernutrition in the experimental animals, due undoubtedly to lowered nutrient consumption, at no time was it certain that any was suffering from phosphorus deficiency in any degree comparable to the condition observed in cattles.[3]

The inorganic phosphorus content of the blood of the animal was the index of phosphorus deficiency used throughout the experiment.

3. It is interesting to note in this connection that J. A. Gilruth, reporting on observations made in Australia, states that a deficiency of phosphorus in the feed is not so vital to rabbits, running wild over the range, as it is to breeding cattle and sheep. Rabbits apparently have a lower requirement for this element or else utilize the phosphorus in their feed to better advantage. (Australian Veterinary Journal 8:162-172. 1932.)

While the most pronounced lowering of this constituent was observed in lactating females, in no instance were the observed changes outside the deviations reported for normal rabbits.

Since, in this preliminary work, it was not found possible to develop symptoms of marked aphosphorosis in rabbits, it was decided to attempt the solution of the problem with dairy cattle. The experiments with dairy cattle reported in this bulletin were conducted at the Kansas Agricultural Experiment Station.

EXPERIMENTAL WORK WITH DAIRY CATTLE

In the majority of experiments reported in the literature concerning aphosphorosis in cattle the actual experimental work has been carried out in areas where the mineral deficiency was prevalent. In the present investigation the work was performed at the Kansas Agricultural Experiment Station in a section of the country where symptoms of phosphorus deficiency had not been recognized. It was anticipated, therefore, that some difficulty might be encountered in obtaining feeds sufficiently low in phosphorus to insure developing the condition in dairy cattle within a reasonable length of time.

LOCATION OF LOW-PHOSPHORUS ROUGHAGE IN KANSAS

Prairie hay is a staple crop in Kansas. Much of the experimental work on phosphorus deficiency at the Minnesota station has involved the use of this feed as the sole roughage in the ration. It was thought that by consulting available soil-survey data *(37)* for this state a possible source of supply from soil areas of low-phosphorus content might be located which would serve the purpose of the present investigation. It was ascertained from the Department of Agronomy that upland soils in the southeastern section of the state would in all probability yield a roughage of lower phosphorus content than any other areas so far surveyed. In September, 1929, a tour was made of several of the counties in this area, and samples of hay collected for analysis. The phosphorus content of these samples is given in Table I, with a description of the soil type on which the sample originated.

A scrutiny of the analyses presented in Table I following reveals the fact that all samples, with the possible exception of No. IV, can be classified as distinctly low in phosphorus. It is interesting to compare these figures with those reported by Eckles and his associates for prairie hay from affected farms in western Minnesota. Three different lots of this hay used in experimental work at the Minnesota station showed an average phosphorus content of 0.09 per cent. Theiler has reported similar figures for hay from badly affected areas in South Africa.

Samples I to VII, inclusive, were collected in counties on the eastern border of the Flint Hills grazing section of Kansas. Woodson county, in which samples III and IV were obtained, is definitely

TABLE I.—PHOSPHORUS CONTENT OF KANSAS PRAIRIE-HAY SAMPLES

Sample No.	County.	Soil type.	Phosphorus.	Remarks.
			Per cent.	
I	Coffey.....	Upland sandy loam....	0.076	Low yield, 50 tons from 80 acres.
II	Coffey.....	Upland sandstone.....	.074	———
III	Woodson...	Upland sandy loam....	.080	———
IV	Woodson...	Sandy loam...........	.156	County agricultural agent reported bone chewing in cattle on this farm.
V	Wilson.....	Oswego sandy loam....	.090	———
VI	Wilson.....	Oswego sandy loam....	.082	———
VII	Wilson.....	Crawford red limestone.	.089	———
VIII	Labette....	Upland light loam.....	.077	———
IX	Labette....	Upland sandy loam....	.063	This farm reported abnormal appetite in cattle.
X	Riley......	Upland light clay loam.	.073	———

a part of this grazing area and is likewise a heavy shipper of prairie hay to the Kansas City market. Sample III was obtained from the barns of one of the largest shippers of prairie hay in the state. There was some uncertainty in regard to sample IV. The county agricultural agent reported that the owner of the farm from which this particular sample was obtained had been complaining of bone chewing in his cattle. When the farm was visited, however, the owner was absent and a sample of hay was secured from his feed lot. Since this particular farm comprised some bottom land, it was entirely possible that the sample in question had originated there, which would serve to explain the appreciably higher phosphorus content.

In a majority of cases the samples originated on farms in which the land was largely given over to grazing or the production of hay. Such crops as were observed growing on the farms were not of a very productive nature. Samples VIII and IX, obtained in Labette county, were obtained on farms that appeared to give as good chances as any of yielding a low-phosphorus hay, as judged by the character of the crops grown, and the subsequent analyses bore out this belief.

The surprising feature of the whole survey was the fact that a sample of prairie hay collected in the dairy barn at this station, to be used as a standard of comparison, showed a lower phosphorus content than any of the samples collected, with one exception. This hay was being used as roughage for a team of mules owned by the Department of Dairy Husbandry and had been cut from upland soil on a farm about five miles west of the college.

VARIETIES OF GRASSES IN THE PRAIRIE HAY

According to information furnished by the Department of Agronomy the grasses predominating in upland prairie hays grown in eastern Kansas are:

Andropogon furcatus (big bluestem).
Andropogon scoparius (little bluestem).
Panisum virgatum (switch grass).
Sorghastrum nutans (Indian grass).

The hay used throughout this investigation was of the upland grass type, and would average 90 per cent or better of the grasses listed.

TABLE II.—PERCENTAGE COMPOSITION OF FEEDS USED IN EXPERIMENT

FEED.	Moisture	Crude protein.	Crude fibre.	Nitrogen-free extract.	Ether extract.	Ash.	Ca.	P.
Prairie hay.........	8.61	4.25	30.83	46.38	2.52	7.41	0.610	0.072
Molasses, cane......	25.13	3.75	....	62.16	.40	8.56	.835	.078
Blood flour.........	9.11	84.25	1.67	.13	.98	3.86	.350	.237
Beet pulp, dried.....	7.90	8.13	18.42	59.31	.58	5.66	.774	.072
Grain mix (corn and oats).........	9.64	10.69	2.99	71.00	3.87	1.81	.130	.323
Sodium phosphate, monobasic.......	....	....	....	....	....	....	....	24.68

RATION USED IN THE EXPERIMENT

The ration finally decided upon, which has been used with little variation throughout the past two and one-half years, during which different phases of this investigation have been in progress, has been comprised of the feeds listed and the analyses of which are presented in Table II.

The figures given in Table II show clearly that the prairie hay, beet pulp, and cane molasses were definitely low phosphorus in composition. The blood flour was really finely ground blood meal and showed a wider range in phosphorus content than any of the other feeds employed. Attention is called to the high-protein content of this feed. While possessing an objectionable odor, no difficulty was experienced in getting the cows to consume the ration containing it, due to the fact that with the small amount required in balancing the ration this undesirable feature, in all probability, was masked by the other feeds.

The grains and molasses were added with the thought of lending as much variety to the ration as possible, and therefore enhancing its palatability. It was readily noted that phosphorus-deficient animals periodically suffered loss of appetite and refused more or less of their feed, depending on the severity of their condition.

A further important reason for securing as much variety in the ration as possible was to exclude any variable factors other than phosphorus which possibly might be introduced through the use of a ration more restricted in character. It is not suggested that this ration was complete in all other essential constitutents, but it is felt that, in addition to meeting the protein and energy requirements of the animals, it was of satisfactory protein quality. Since the cows were maintained in dry lot, receiving a full measure of insolation during most of the year, the ration probably supplied the vitamin requirements of the animal reasonably well.

Calcium and Phosphorus Content of the Ration.—The typical ration furnished at the outset of the experiment was as follows: Prairie hay, 10 pounds; beet pulp (dry), 7 pounds; molasses, 3 pounds; blood flour, 1.6 pounds; grain mix (corn and oats), 3 pounds. It contained approximately 0.57 per cent calcium and 0.12 per cent phosphorus. With the phosphorus supplement (100 grams monosodium phosphate) added, the phosphorus content was raised to 0.34 per cent. While the calcium content of this ration was not high, it was probably adequate, but it is quite apparent that the phosphorus was present in subnormal amount. McCollum and his associates *(30)* have reported that the optimum concentration of these minerals in the ration for growing rats is 0.64 per cent calcium and 0.41 per cent phosphorus, respectively. Considering the fact that the rat is a faster growing animal than the dairy cow, with a consequent heavier proportionate demand for these minerals, the present ration probably proved adequate in the supplemented form.

ANIMALS USED IN THE DIGESTION EXPERIMENT

In September, 1929, three grade Holsteins were selected in the herd of a dairyman living near Manhattan and purchased for this experiment. Following removal to the college they were held in the veterinary hospital until the middle of October, when they were pronounced negative to the blood test for Bang's disease and moved into experimental stalls in the dairy barn.

These animals had been on upland pasture all summer and had been moved to the owner's farm about a week before they were purchased. They carried only a fair amount of flesh and were considerably below normal in weight and height for their respective ages.

On removal to the dairy experimental barn, the cows were gradually accustomed to the low-phosphorus ration. Since it was desirable to have as hearty feeders as possible in the low-phosphorus group, the three animals were placed under observation for about a month as to feeding ability, and then selected for the purposes of the experiment.

The final division was as follows:

Control cow, E-7 (receiving phosphorus supplement): A three-year-old, weight 885 pounds, freshened September 14, 1929. A fairly good producer that averaged approximately 30 pounds of milk a day when started on experiment. This cow showed a nervous disposition and during the preliminary observation period showed herself to be a more uncertain feeder. For these reasons she was selected to serve as the control in the present experiment. (Fig. 1.)

Low-phosphorus cow, E-8: A two-year-old, weight 845 pounds, freshened September 26, 1929, averaging approximately 32 pounds daily when placed on experiment.

Low-phosphorus cow, E-9: A two-year-old, weight 834 pounds, freshened October 7, 1929. The outstanding producer of the lot, averaging approximately 40 pounds daily at the start of the experiment. (Fig. 2.)

Feeding and Management of Animals.—The cows were maintained in separate stalls of the Graves type in the experimental barn. With the exception of the colder months, and weather permitting, the cows were in dry lot most of the time, being turned into the barn for feeding and milking. They were fed twice a day. The beet pulp for each day's feeding was soaked the day before with water to which the molasses had been added. It was felt that the soaked beet pulp would be more palatable, but, apparently, after the animal's appetite began to fail, it offered too much bulk, and better success resulted from feeding in the dry form and adding the molasses to the ration in somewhat diluted form. All feed refused was weighed back and a record kept. Water and salt were available at all times, both in barn and dry lot. The salt was furnished in the brick form. Shavings were used as bedding, since it was felt that the use of the more common straw bedding might introduce another variable.

Weights of the animals involved, with measurements of height at withers, were taken at monthly intervals on the last three days of each month. Daily milk weights were recorded for each cow. The butter-fat content was determined monthly on a five-day composite sample of each cow's milk, taken about the middle of the month.

Close observation was maintained on the experimental animals throughout the course of the investigation, and symptoms of abnormal appetite or behavior carefully noted.

PHASE I

DIGESTION, NITROGEN, AND MINERAL-BALANCE TRIALS

The general plan of this phase of the investigation was as follows:

1. Preliminary period of feeding on the low-phosphorus ration until symptoms of phosphorus deficiency were evident.

2. Period of feeding in metabolism stalls during which the digestibility of the ration was determined and the nitrogen and mineral balances of the animals secured.

Since the digestibility determinations were to be made on animals in the phosphorus-deficient condition, the major concern was to induce aphosphorosis in the experimental animals through feeding the low-phosphorus ration. One cow, E-7, as already indicated, was to serve as control throughout the experiment and received the phosphorus supplement.

As the problem under investigation was that of the relation of phosphorus intake to the utilization of feed, it was desirable that the plane of nutrition be made uniform. These animals, therefore, received a uniform intake of 110 per cent of the estimated digestible nutrients required as prescribed by the Morrison feeding standard. This intake was adjusted at the beginning of each month to meet changes in the milk production and live weight of the animals involved or as the condition of the experiment called for.

In calculating the digestible nutrients in the feeds used, the digestion coefficients as given in Henry and Morrison's table of average digestibility of American feeding stuffs *(26)* were used.

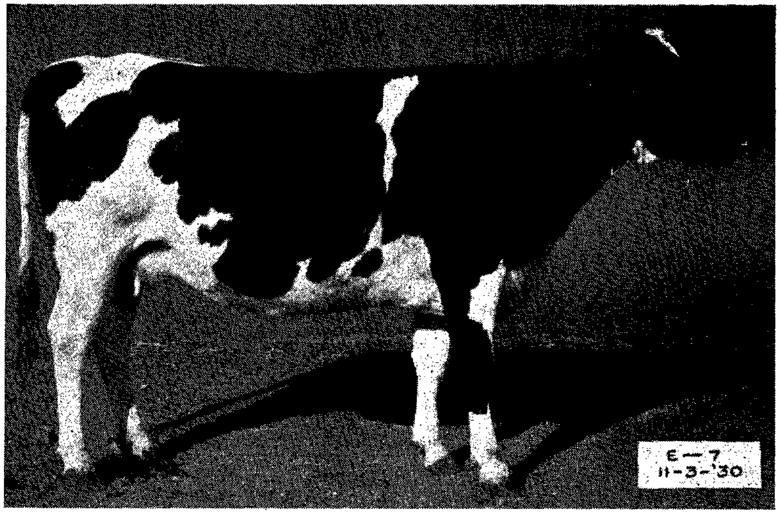

FIG. 1.—Control cow, E-7, after one year on experimental ration plus phosphorus supplement, during which time she gained 305 pounds.

Observations Made During the Preliminary Period

The three cows selected for this phase of the investigation were started on experiment the last of October, 1929, and were accustomed to the low-phosphorus ration within a week's time. After a period of approximately one month's observation on the low-phosphorus diet, the cows were selected as to appetite and general reaction to the ration. The cow E-7 (fig. 1), which was finally selected as the control, proved a temperamental sort of feeder, while E-8, and especially E-9 (fig. 2), responded to the ration in a much more satisfactory manner. Thus, strictly speaking, while E-8 and E-9 received the low-phosphorus ration commencing November 1, E-7, the control cow, did not begin to serve as control until the start of the following month, when the phosphorus supplement was supplied in the ration.

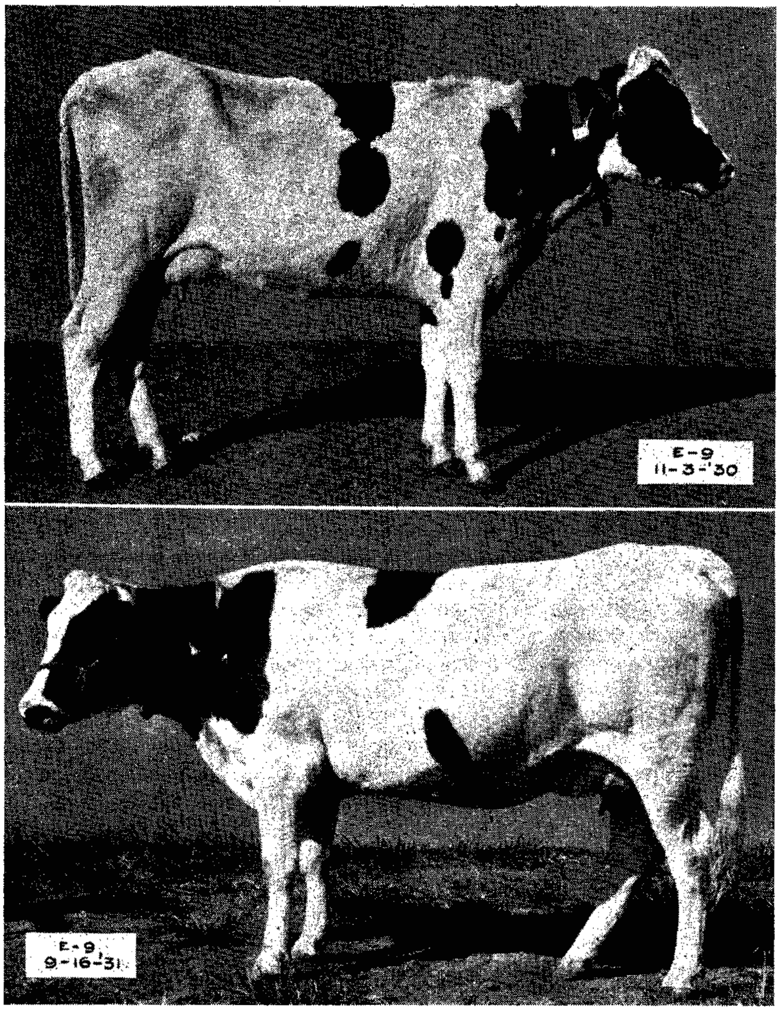

FIG. 2.—Upper—Cow E-9 after one year on the low-phosphorus experimental ration. In spite of the fact that she was in poor flesh at the start and received the phosphorus supplement for short periods, she lost approximately 100 pounds in weight during the year. Lower—the same cow after receiving phosphorus supplement for 10 months and gaining 353 pounds.

FAILURE OF APPETITE

Throughout November and December all three cows consumed their rations in a satisfactory manner. In January, however, the first symptoms of diminished appetite began to appear in E-8 and E-9, the low-phosphorus cows. Feed would be refused periodically. The amount refused was small at first, but gradually increased with each lapse of appetite. By the end of February appreciable quantities of feed were being weighed back for each of these low-phosphorus cows. No difficulty in this matter of feed consumption was experienced at any time with the control cow, E-7, receiving the phosphorus supplement. As a result, this periodic failure of appetite, which became more pronounced as the experiment progressed, can undoubtedly be explained by the deficiency of phosphorus in the ration. Theiler *(40)* and Du Toit *(14)* have reported that phosphorus is a limiting factor in voluntary food consumption, while Evans *(20)*, working with swine, noted similar loss of appetite in feeding rations low in calcium, which he attributed to the deficiency of lime.

A marked improvement in appetite followed the introduction of phosphorus supplement into the ration of the deficient animals. The response was immediate in practically all cases observed. This emphasizes an important relationship between the level of phosphorus intake and its marked effect on appetite.

DEPRAVED APPETITE AND STIFFNESS

The months during this preliminary period were colder than the average for Kansas, and the cows were kept in the barn with only a brief period or two in the exercise yard. In addition to the failure of appetite already noted, the cows E-8 and E-9 developed a craving for shavings, which formed their bedding. Early in January E-9 was observed eating shavings after approximately 70 days on the low-phosphorus ration. This craving was not observed in E-8 until early in the following month. Since this condition was not noted in the control, it was regarded as an early symptom of depraved appetite, and it was significant that both cows showed it in more pronounced degree as the experiment advanced.

About the middle of February, after approximately three and a half months on the experimental ration, E-9 began to show evidence of stiffness in her gait and by the end of the month was pronounced quite stiff. This condition showed particularly in her hind quarters, with a marked tendency to drag her hind legs. Early in March she began to experience difficulty in rising to her feet, and on March 8 it was decided to add the phosphorus supplement to her ration, before she became too weak. Accordingly 100 grams of monosodium phosphate were supplied daily. The immediate response in appetite and the early improvement in the general reaction of this cow furnished abundant evidence of the benefit derived from the increased phosphorus intake. After receiving the phosphorus supplement for one week the following statement was made in the record of the experiment, "A different animal, brighter eyes, more alert, though still stiff in gait." At the end of two weeks it was noted that E-9 was walking better, general reaction more alert.

The characteristic stiffness of hind quarters did not become marked in E-8 until approximately two weeks later, and on March 19 it was deemed advisable to feed the phosphorus supplement.

This cow evidently had been weakened to a greater extent than E-9, for after one week's feeding of the supplement it was noted that she was still too weak to lead to the scales, and the rate of phosphate feeding was increased to 150 grams daily. By the end of the month considerable improvement was noted, and the rate of phosphate feeding was reduced. Early in March this cow, also, was observed eating dirt and chewing on rocks in the exercise lot.

RELATION OF PHOSPHORUS INTAKE TO GAIN IN WEIGHT

A consideration of the results, from the standpoint of the gains in weight involved, brings out even more striking differences between the normal and low-phosphorus cows. These data are presented in Table III.

TABLE III.—THE RELATION OF PHOSPHORUS INTAKE TO DAILY GAIN IN WEIGHT

Cow.	Ration.	Plane of nutrition.	Length of period.	Average daily gain.	Average daily milk yield.	Phosphorus per day.
		Per cent.	*Days.*	*Lbs.*	*Lbs.*	*Gm.*
E-7....	Basal+NaH_2PO_4........	110	90	+0.4	23	39.8
E-8....	Basal.................	110	120	—.6	25	13.7
E-9....	Basal.................	110	120	—.8	28	14.2

The results given in Table III cover the period, November 1 to March 1, inclusive, for the low-phosphorus cows E-8 and E-9 and starting a month later for the control cow E-7, since she did not receive the phosphorus supplement until that date. Had this cow received the normal ration at the outset, it seems reasonable to expect that she would have shown a more substantial gain in weight than was observed. The 30-day period on the low-phosphorus ration undoubtedly had an influence on the later gains in weight which this cow was able to make. As it is, her average daily gain of 0.4 pound is in marked contrast to the average daily losses in weight registered by E-8 and E-9, 0.6 and 0.8 pound, respectively, on the same uniform plane of nutrition. Eckles and Gullickson *(17)* have reported that a plane of feeding of at least 120 per cent was necessary to even maintain the live weight of phosphorus-deficient animals.

Attention is directed to the good average daily milk yield of the three cows involved. The demand for phosphorus to maintain this produotion, with the consequent rapid depletion of the phosphorus reserves of the animal, was undoubtedly the determining factor in producing the symptoms of phosphorus deficit as rapidly as the records of the experiment indicate. Eckles, Becker, and Palmer *(16)* reported the production of depraved appetite in 118 days with cattle under controlled conditions, but the milk production of their cows did not average nearly so high as in the present investigation.

Milk and Butter-fat Production of Experimental Cows.— The manner in which milk production was maintained in the low-phosphorus cows was a matter of considerable interest. The monthly milk and butter-fat production of the experimental cows is given in Table IV for the period October, 1929, to September, 1930, inclusive. Even after four months of the phosphorus-deficient ration, when these cows became so stiff and in such a weakened condition that they could rise only with difficulty, their average daily milk production was well maintained. It was remarkable the way these cows persisted in production in the face of such difficulties. Theirs must have been an unusually good inheritance, particularly in the case of E-9, for they both proved themselves better producers than the control cow.

TABLE IV.—MILK AND BUTTER-FAT PRODUCTION OF EXPERIMENTAL COWS

MONTH.	E-7, control (a).			E-8, low-P (a).			E-9, low-P (a).		
	Milk.	Fat.	Fat.	Milk.	Fat.	Fat.	Milk.	Fat.	Fat.
	Lbs.	*Per cent.*	*Lbs.*	*Lbs.*	*Per cent.*	*Lbs.*	*Lbs.*	*Per cent.*	*Lbs.*
1929									
Oct.	691.0	3.6	24.88	668.4	2.5	16.71	685.1	3.8	26.03
Nov.	757.1	3.7	28.01	845.9	2.5	21.15	961.2	3.4	32.68
Dec.	671.7	3.2	21.49	793.1	3.0	23.79	896.1	3.3	29.57
1930									
Jan.	737.6	3.5	25.82	739.3	3.0	22.18	812.1	3.2	25.99
Feb.	651.1	3.6	24.44	667.9	3.1	20.70	709.6	3.3	23.42
Mar. (b)	769.9	3.6	27.72	718.7	3.4	24.44	764.3	3.8	29.04
April	729.8	3.3	24.08	805.0	3.5	28.18	854.9	3.4	29.07
May (b)	728.8	3.3	24.05	842.7	2.9	24.44	852.8	3.0	25.58
June	550.7	2.9	16.19	720.3	2.4	17.14	702.8	3.2	22.42
July	534.6	3.0	16.20	653.4	2.4	15.62	625.8	3.0	19.02
Aug.	447.8	3.6	16.12	641.9	3.0	19.26	649.0	3.0	19.47
Sept. (c)	114.2	3.6	4.11	542.3	3.4	18.44	618.0	3.8	23.48

(a) E-7 freshened Sept. 14; E-8, Sept. 26; and E-9, Oct. 7, 1929.
(b) Phosphorus supplement fed to low-phosphorus cows during periods, March 20 to April 27 and May 16 to June 7.
(c) Phosphorus supplement also fed to E-8 and E-9, Sept. 5 to 8, and to E-9, Sept. 26 to 30, 1930. E-8 died Sept. 26, as a result of sugar injection.

Attention is directed to the marked response in production in the low-phosphorus cows, when phosphorus supplement was introduced into their rations the latter part of March after more than five months on the phosphorus-deficient ration. A substantial increase in butter-fat percentage was noted, also, in both cows, which was not observed in the control.

While E-7 and E-8 terminated their lactations in September, 1930, E-9 continued in production until June of the following year. Her total production for the first 365 days of her lactation was 9,879 pounds of milk containing 334 pounds of butter fat. When it is considered that, except for the relatively short periods of phosphorus feeding indicated, she was on the phosphorus-deficient ration all of this time, the evidence would indicate that phosphorus deficiency did not play so important a role in limiting milk production as it did in body increase.

LIVE WEIGHT AND FEED CONSUMPTION

In Table V is presented a summary of the data concerning the feed consumption and live weight of the experimental animals during the period preceding the digestion trials. This covers the period November 1, 1929, to June 17, 1930, inclusive. In an even more striking manner than was possible in Table III, is shown the marked reaction of the low-phosphorus cows, E-8 and E-9, to an increase or decrease in the level of phosphorus intake. It is well to recall that the three experimental animals were fed on a plane of feeding of 110 per cent of the estimated digestible nutrients required. It was certain that they had an ample supply of nutrients to meet the requirements of maintenance and milk production and allow for some increase in body weight. This was evident from the substantial gain in weight made by the control, E-7. In marked contrast was the behavior of the low-phosphorus cows, each of which showed a decrease in live weight for the period, November to March,

TABLE V.—LIVE WEIGHT AND FEED CONSUMPTION OF COWS DURING THE PRELIMINARY PERIOD

Cow.	Period.	Live weight.	Approximate digestible nutrients.	
			Consumed.	Refused.
		Lbs.	*Lbs.*	*Lbs.*
E-7 (control)	Nov. 1, 1929	907	....	....
	Nov. 1-30, 1929	885	492	0
	Dec. 1-31, 1929	875	473	0
	Jan. 1-31, 1930	885	495	0
	Feb. 1-28, 1930	922	455	0
	March 1-31, 1930	935	517	0
	April 1-30, 1930	974	510	0
	May 1-31, 1930	990	524	0
	June 1-17, 1930	977	263	0
E-8 (low-P)	Nov. 1, 1929	845	....	....
	Nov. 1-30, 1929	849	492	0
	Dec. 1-31, 1929	840	473	0
	Jan. 1-31, 1930	800	425	40
	Feb. 1-28, 1930	762	289	102
	March 1-19, 1930	753	233	33
	Mar. 20—Apr. 27 (*a*)	841	574	9
	April 28—May 15	805	274	16
	May 16—June 7 (*a*)	838	253	0
	June 8-17, 1930	819	155	0
E-9 (low-P)	Nov. 1, 1929	834	....	....
	Nov. 1-30, 1929	855	492	0
	Dec. 1-31, 1929	850	552	0
	Jan. 1-31, 1930	786	491	20
	Feb. 1-28, 1930	735	394	23
	March 1-8, 1930	735	118	0
	Mar. 9—Apr. 27 (*a*)	785	766	27
	April 28—May 15	764	258	70
	May 16—June 7 (*a*)	777	379	0
	June 8-17, 1930	773	151	13

(*a*) Phosphorus supplement fed throughout this period.

of approximately 100 pounds. When the phosphorus supplement was added to their rations in March, E-8 over a period of 39 days registered a net gain of 88 pounds, or more than a 2-pound daily increase in body weight. With E-9 the results were not so outstanding but, nevertheless, emphasized in a forceful manner the marked influence that the level of phosphorus intake has on feed utilization, as reflected in the animal's live weight. Furthermore, when phosphorus feeding was discontinued for the two cows on April 27, and not resumed again until May 16, they showed a decrease in live weight of 36 and 21 pounds, respectively, for the 18-day period. During the subsequent 23-day period of phosphorus feeding, substantial gains were again registered by both cows.

The effect, of progressive aphosphorosis on the animal's appetite has already been commented upon. It can readily be seen from the data in Table V that, while some of the loss in weight can be laid to the periodical failure of appetite of the animals involved, the major cause lies elsewhere. For the period November to March the digestible nutrients refused by the low-phosphorus cows, E-8 and E-9, represented 9.2 per cent and 2.1 per cent, respectively, of their total intake. It was certain that they were consuming sufficient nutrients to produce some gain in weight had the ration been adequate in phosphorus content. This resulted when the phosphorus supplement was included in the ration.

INFLUENCE OF PHOSPHORUS DEFICIENCY ON THE BLOOD

Palmer and Eckles *(33)* in this country, and Theiler, Malan, and associates *(31, 41)* in South Africa, have demonstrated that the outstanding characteristic of the blood of animals in a state of phosphorus deficiency is its low inorganic phosphorus content. It has been found that this fraction of the blood may drop to a quarter of the normal value, even before the disease can be diagnosed clinically. In view of the close association of calcium and phosphorus in mineral metabolism, it is interesting, also, to note that the calcium content is unaffected by the marked reduction in phosphorus.

In the present investigation it was planned to check symptoms of aphosphorosis with analyses on the blood. Inorganic phosphorus was determined by the method of Fiske and Subbarrow *(22),* while blood calcium was determined by the Kramer and Tisdall *(29)* method on a single day's sample of blood drawn in the beginning of each month noted. The data are presented in Table VI.

The data in Table VI clearly furnish additional proof that the cows E-8 and E-9 were in a state of phosphorus deficiency early in February, which became more marked by the following month. It is regretted now that earlier determinations were not obtained marking the gradual reduction in concentration of the inorganic phosphorus of the blood. These animals showed symptoms of aphosphorosis a good deal earlier than had been anticipated, and it is felt that the good milk production of the cows involved, together with the low-phosphorus character of the ration, has been responsible, in

TABLE VI.—INFLUENCE OF PHOSPHORUS DEFICIENCY ON THE BLOOD

ANIMAL	Inorganic phosphorus (mg. per 100 c. c. whole blood).			Calcium (mg. per 100 c. c. plasma).		
	Feb.	Mar.	Apr.	Feb.	Mar.	Apr.
Normal cow E-7	3.73	5.60	5.44	12.30	13.4	12.5
E-8 (low-P)	1.35	.96	(a) 6.24	12.67	16.7	12.0
E-9 (low-P)	.94	.57	(a) 5.48	12.60	13.8	13.5

(a) This analysis made after E-8 and E-9 had been receiving phosphorus supplement for 14 and 25 days, respectively.

the main, for the early indications of the disorder. This also would help to explain the very low phosphorus values observed. It will be noted that the values for plasma calcium remain normal or even increase somewhat.

REPRODUCTION OF EXPERIMENTAL ANIMALS

There is considerable evidence in the literature on the effect of a deficient phosphorus supply on reproduction. This has been adequately reviewed by Theiler and Green *(38)*. A frequent observation has been the failure of œstrum in affected animals. This was demonstrated rather strikingly in the present study. The three cows were started on experiment November 1, 1929. The control, E-7, receiving the phosphorus supplement, showed œstrum regularly until she was bred on March 22, 1930. She calved in a normal manner on December 24, 1930.

The two low-phosphorus cows behaved quite differently, E-8, although receiving phosphorus supplement for several periods of the experiment, as indicated in Table V, showed no manifestation of heat in the 11 months that she was maintained on the low-phosphorus ration. The collection of further data on this interesting phase of phosphorus deficiency was prevented only by her death September 26, 1930, as the result of a sugar-injection experiment carried out on her at the time. With E-9 the behavior was very similar. Œstrus was not recognized in this cow in more than a year's time. She was returned permanently to an adequate phosphorus ration early in December, 1930, after receiving phosphorus supplement for several short periods in the two preceding months. Her first observed œstrum occurred December 16, 1930. She was bred the following month and calved normally October 10, 1931.

These observations are in accord with those of Eckles, Becker, and Palmer *(16)* that the most severe cases of failure in reproduction among cows on low-phosphorus rations were the heavy-producing animals. The heavy drain of milk production upon the mineral supply of the body is apparently manifested in a comparatively short time in the suspension of ovulation, which is resumed only when this depleted mineral reserve has been restored.

Digestion Trial Period

The digestion trials were not run until the latter part of June, 1930. In the interval between the first marked symptoms of aphosphorosis in the experimental animals and the period of the trials, the low-phosphorus cows had received phosphorus supplement on two occasions over extended periods, and it was not until the first part of June that they were judged to be again in a suitable condition of phosphorus deficit. Proof of this was furnished by the symptoms of depraved appetite, general lack of thrift, and, also, by the blood analysis made at the conclusion of these trials. While the control cow, E-7, showed an inorganic phosphorus content of 8.72 mg. per 100 c. c. of whole blood, E-8 ann E-9 showed determinations of 1.34 mg. and 1.2 mg., respectively.

The cows were placed in the metabolism stalls June 17, and the collection period was begun on June 28 at 7 a. m. and concluded the morning of July 8.

In order to secure complete consumption of feed during the trials, the ration was finally adjusted as follows: Prairie hay, 4 pounds; beet pulp (dry), 4 pounds; molasses, 2 pounds; blood flour, 1.2 pounds; grain mix (corn and oats), 7 pounds. The same ration was furnished the three cows, which lessened the chances for error in the actual weighing of each day's feed and facilitated the final calculations. This ration proved adequate for the requirements of maintenance and milk production for the low-phosphorus cows, but was below the requirements for the normal cow, E-7. The final results, however, did not indicate that the general outcome of the trials was influenced by this procedure.

Sampling of Feeds.—A sufficient supply of the several feeds employed in these trials was set aside, and a uniform sample of each secured for analysis at the outset of the experiment. The supply of prairie hay was cut, mixed, and sampled for the whole trial at one time. All feeds were weighed separately on gram scales for each feeding. The methods of analysis were essentially those recommended by the Association of Official Agricultural Chemists.

Management of Animals.—The metabolism stalls were built from plans furnished through the courtesy of the Department of Animal Industry of the University of Vermont. The cows stood practically at barn-floor level on a heavy grade of waterproof canvas, which was underlaid in turn with rubber matting and several inches of shavings, The essential feature of these stalls was a large tin apron inclosing the rear end of the stall and extending sufficiently well behind the cow to catch and direct all liquid excrement through a funnel-shaped opening into a container set in the floor below.

The mangers were sufficiently deep to insure against scattering of feed by the cow. As an additional precaution a canvas curtain suspended from a ring was let down around each cow's head at feeding time. Since ample time was provided in the adjustment period for the animals to become accustomed to their new conditions before the actual collection was started, no difficulty was encountered from any of these sources.

The cows were fed and milked on strict schedule, and all conditions relating to the experiment were carefully controlled. Water was furnished three times a day, and an accurate record of consumption by each cow was obtained. This water came from the college supply and contained a trace of calcium (0.008 per cent), but was found free of phosphorus. Body weights of the cows were taken at the beginning and end of the trials.

Collection of Excreta.—Attendants were on duty twenty-four hours each day. Specially constructed pails, with wooden handles attached at right angles to the mouth of the pail, were used in catching all excrement. The particular type of metabolism stall employed served as an added precaution against any loss or contamination from outside sources. Each voiding was placed in a can provided for each cow for the purpose. Since the weather was quite warm throughout the duration of the trials, toluene was used as a preservative for both the urine and fecal excreta.

In the morning, at the end of each 24-hour period, each cow's excreta was weighed and a representative sample taken for analysis. This sample represented 5 per cent of the feces and 2 per cent of the urine, respectively. The milk composite, comprising 2.5 per cent of each cow's yield, was preserved with formalin and kept in an ice box, as were the samples of urine. Each day's samples of feces for each cow were taken to the chemistry department, added to the composite for the trial, and dried to constant weight.

The data obtained in the digestion trials are presented in Tables VII and VIII. The analysis of feeds used in these trials appears in Table II.

TABLE VII.—PERCENTAGE COMPOSITION OF FECES

Cow.	Voided.	Crude protein.	Crude fiber.	Nitrogen-free extract.	Ether extract.	Ash.	Calcium.	Phosphorus.
	Gm.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*
E-7......	23,261	21.56	17.48	42.02	2.36	12.92	1.16	1.098
E-8......	21,525	22.69	17.29	42.95	2.16	10.72	1.26	.480
E-9......	24,294	20.94	18.66	43.09	2.28	9.95	1.24	.419

The authors desire to express their indebtedness to Prof. W. L. Latshaw, in charge of the analytical laboratories, for the analytical work involved in the digestion and balance trials and for many helpful suggestions regarding the collection and sampling of feeds and excreta for analysis.

TABLE VIII.—AMOUNTS OF THE DIFFERENT NUTRIENTS CONSUMED, VOIDED, AND APPARENTLY DIGESTED BY EACH COW DURING THE TRIAL

	Dry matter.	Crude protein.	Crude fiber.	N-free extract.	Ether extract.
Nutrients Consumed (Grams)					
Prairie hay	16,596	772	5,599	8,423	458
Beet pulp	16,725	1,476	3,345	10,771	105
Molasses	6,798	341	....	5,644	36
Blood flour	4,952	4,590	91	7	43
Corn and oats	28,716	3,397	950	22,564	1,330
Totals	73,787	10,576	9,985	47,409	1,972
Nutrients Voided in Feces (Grams)					
Cow:					
E-7 (control)	22,440	5,015	4,066	9,774	549
E-8 (low-P)	20,660	4,884	3,722	9,245	465
E-9 (low-P)	23,320	5,087	4,533	10,469	554
Nutrients Apparently Digested (Grams)					
E-7 (control)	51,348	5,561	5,919	37,634	1,433
E-8 (low-P)	53,128	5,692	6,263	38,163	1,517
E-9 (low-P)	50,468	5,489	5,452	36,940	1,429
Coefficients of Digestibility (Per Cent)					
E-7 (control)	69.6	52.6	59.3	79.4	72.3
E-8 (low-P)	72.0	53.8	62.7	80.5	76.5
E-9 (low-P)	68.4	51.9	54.6	77.9	72.1
Average, low-P cows	70.2	52.8	58.6	79.2	74.3

Discussion of Data from Digestion Trials

The amounts of the different nutrients consumed, voided, and apparently digested by each cow, together with the observed coefficients of digestibility are shown in Table VIII. The digestion coefficients indicate that the ration employed in the trials was of average digestibility. The digestion coefficient for the crude protein was the lowest of any of the nutrients studied. This fact undoubtedly can be explained by the low order of digestibility of this nutrient in three of the feeds which formed so large a part of the ration; namely, the prairie hay, beet pulp, and molasses.

It will be observed that the coefficients of digestibility for the carbohydrate portion of the ration, which includes by far the largest percentage of the nutrients consumed, show very close agreement for the three animals. This also can be said of the crude protein. For the other nutrients the variations are not sufficiently marked to be regarded as outside the limits of experimental error in making determinations of this kind.

The reasonably close agreement between the digestion coefficients for the three animals indicates that digestion is not impaired when the animal is in the phosphorus-deficient condition. The observed differences between the low-phosphorus animals and the control are not sufficiently marked or consistent to be regarded as significant. While at first sight it appears that the low-phosphorus cow, E-8, has digested her feed more efficiently than the control, on the other hand the reverse holds true for the other member of the pair, E-9. In such a comparison as the present, based on a single digestion trial with three animals, the results may prove misleading, and it is felt that the observed differences are, in a sense, more accidental in nature than due to any actual difference in digestive efficiency of the animals involved. In view of the fact that both low-phosphorus cows were, relatively, in approximately the same stage of aphosphorosis, it is felt that the mean of the digestion coefficients for these animals more nearly represents their actual digestive ability. These figures are given in Table VIII, and it will be observed that the average coefficients for these two cows show so small a variation from the observed values for the control as to support the conclusion that phosphorus deficiency does not influence the animal's ability to digest its feed.

When the fact is considered that digestion is promoted largely by bacterial and enzymatic action, and that phosphorus is required in very small amounts in these processes, there is probably little reason why a deficiency of this element in the ration should depress digestibility. What is lacking in the ration is, undoubtedly, made up in liberal amount from the mobile reserves of the animal.

TABLE IX.—PERCENTAGE COMPOSITION OF URINE

Cow.	Voided.	Moisture.	N.	Ash.	Ca.	P.	Specific gravity.
	Gm.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
E-7 (control)	70,635	95.17	0.659	2.16	0.005	0.0188	1.0317
E-8 (low-P)	82,023	96.02	.536	1.98	.061	.0013	1.0278
E-9 (low-P)	98,103	97.07	.387	1.55	.041	.0012	1.0222

Water consumed (gm.): E-7, 346,620; E-8, 353,224; E-9, 367,876.

TABLE X.—PERCENTAGE COMPOSITION OF MILK

Cow.	Produced.	Moisture.	N.	Ca.	P.	Specific gravity.
	Gm.	*Per cent.*	*Per cent.*	*Per cent.*	*Per cent.*	
E-7 (control)	80,240	88.13	0.488	0.097	0.095	1.0335
E-8 (low-P)	100,210	89.13	.422	.097	.084	1.0317
E-9 (low-P)	96,792	89.15	.462	.092	.084	1.0322

TABLE XI.—AVERAGE DAILY NITROGEN, CALCIUM, AND PHOSPHORUS BALANCES OF EXPERIMENTAL ANIMALS DURING DIGESTION PERIOD

Weight in grams.

Cow.	Nitrogen in—			Nitrogen.		NITROGEN BALANCE.
	Feces.	Urine.	Milk.	Outgo.	Intake.	
E-7 (control)	80.2	46.6	39.2	166.0	169.2	+3.2
E-8 (low-P)	78.2	43.9	42.3	164.4	169.2	+4.8
E-9 (low-P)	81.4	37.9	44.7	164.0	169.2	+5.2
Cow.	**Calcium in—**			**Calcium.**		**CALCIUM BALANCE.**
	Feces.	Urine.	Milk.	Outgo.	Intake.	
E-7 (control)	26.9	0.4	7.8	35.1	41.5	+6.4
E-8 (low-P)	27.1	5.0	9.7	41.8	41.5	—0.3
E-9 (low-P)	30.1	4.0	8.9	43.0	41.7	—1.3
Cow.	**Phosphorus in—**			**Phosphorus.**		**PHOSPHORUS BALANCE.**
	Feces.	Urine.	Milk.	Outgo.	Intake.	
E-7 (control)	25.5	1.3	7.6	34.4	39.6	+5.2
E-8 (low-P)	10.3	.1	8.5	18.9	14.9	—4.0
E-9 (low-P)	10.2	.1	8.1	18.4	14.9	—3.5

Discussion of Nitrogen and Mineral-Balance Data

The data relating to the nitrogen and mineral metabolism of the experimental cows are given in Tables IX, X, and XI. The analyses of feeds used and feces voided have already been noted in Tables II and VII, respectively.

Since positive nitrogen balances were obtained for the normal and low-phosphorus cows, it would indicate that the protein of the ration met their nitrogen requirements in a satisfactory manner. The nitrogen retention was not marked in any of the animals, but higher figures were obtained for the phosphorus-deficient cows, E-8 and E-9, than for E-7, the control. This fact was reflected in the record of their live weights during the 10-day period of the digestion trial. While E-7 just failed of maintaining her live weight, with a decrease of 4 pounds, E-8 and E-9 registered small gains of 5 and 4 pounds, respectively. These figures are well within the limit of experimental error in weighing, and when it is considered that all three cows had lost in weight from the time they were placed in the metabolism stalls until the start of the collection period, because of reduction in the amount of ration fed and adjustment to new conditions, the small positive nitrogen balances noted would indicate that nitrogen equilibrium had been attained and some storage was taking place.

A consideration of the mineral balance data, as summarized in Table XI, brings out quite clearly the influence of the level of phosphorus intake on the calcium and phosphorus metabolism in the experimental animals. While E-8 and E-9, on a phosphorus intake of 14.9 grams daily as contrasted with 39.6 grams for E-7, showed negative daily balances of 4 grams and 3.5 grams, respectively, a positive balance of 5.2 grams daily was recorded for the control. It must be concluded, therefore, that the ration, while falling considerably short of the phosphorus requirements of the deficient cows, proved adequate in the supplemented form and allowed for some storage. The early occurrence of depraved appetite and other symptoms of aphosphorosis on this ration, throughout different phases of the present investigation, are explained in large measure by the striking character of the balances obtained. The depletion of phosphorus reserves must have progressed at a rapid rate in the low-phosphorus cows, aided materially by their high average level of milk production.

It is interesting to note the remarkable uniformity of results for the different paths of outgo in the low-phosphorus cows. Apparently depletion of mineral reserves had reached approximately the same stage for each of these cows at the time of the trial. Attention is also called to the greater urinary excretion of phosphorus in the case of E-7. The urine of the low-phosphorus cows, on the other hand, was extremely low in this mineral. This further emphasizes the extent to which aphosphorosis must have proceeded in cows E-8 and E-9. Since the urinary phosphorus has come from the blood, the low amounts in the urine are significant in view of the low concentration of this mineral in the blood of these animals, as determined at the close of the trial and noted elsewhere.

The close association of calcium and phosphorus in metabolism is well illustrated in the data on the calcium and phosphorus balances. It will be observed that the negative phosphorus balances in E-8 and E-9 were accompanied by calcium balances of similar character, though not so marked in extent. On the other hand, the control, E-7, showed considerable storage of these minerals during the trial. It is evident that the heavy demand for phosphorus in the deficient animals has resulted in a breakdown of skeletal material, with consequent elimination of calcium from the body in the urine, to a large extent.

Attention is directed to the data in Table X on the percentage composition of the milk produced by the experimental animals. Becker, Eckles, and Palmer *(3)* have reported that even under conditions of extreme and long continued shortage of phosphorus the calcium and phosphorus content of the milk remained normal in amount and proportion. In the present experiment it is interesting to note that both of the low-phosphorus cows produced milk of a lower phosphorus content than the control. In view of the limited number of animals concerned, it is doubtful if any significance can be attached to this observation. More likely it is due to chance and the individuality of the animals.

PHASE II

DETERMINATION OF METABOLIZABLE ENERGY

Since the digestion study showed that the low-phosphorus cows were digesting the nutrients of the ration satisfactorily, it was necessary to look elsewhere for explanation of the lowered effectiveness in feed utilization of the deficient cows. In view of the important role of phosphorus in body oxidations it was possible that in the phosphorus-deficient condition there was a failure to carry the oxidation of the assimilated nutrients to completion. It was, therefore, decided to determine whether abnormal energy losses could be demonstrated in the excreta of the low-phosphorus animals. The heat of combustion values of the feeds consumed and the visible excreta were determined. By estimating the losses of energy in the gases produced as a result of fermentation in the intestinal tract, it was possible to calculate the metabolizable energy of the ration fed.

ANALYTICAL PROCEDURE

Gross energy determinations were made on feed, urine, and feces by means of an Emerson bomb calorimeter. Values for all feed and feces materials were determined on 1-gram samples according to standard methods.

The samples of milk and urine were prepared for combustion by drying in a platinum crucible under vacuum and over concentrated sulphuric acid. Since decomposition takes place during the drying of urine, producing losses of energy, it was necessary to make corrections for these losses. According to Braman *(7)*, the decomposition of ammonium carbonate is largely responsible. Therefore, the loss of nitrogen during drying of the sample was determined and a correction made according to information furnished by Forbes in a private communication (energy per gram of nitrogen lost = 5,447 calories).

While it was possible to measure satisfactorily the losses of energy in the feces and urine of the experimental animals, it was necessary to compute the losses, chiefly methane, arising from the fermentation of carbohydrates in the digestive tract. This was done according to the method suggested by Armsby *(1)*, recognizing the fact "that the amount of methane produced is, in general, proportional to the amount of total carbohydrates digested."

The data concerned in the determination of metabolizable energy of the ration used in the digestion trial are presented in Table XII.

TABLE XII.—DATA USED IN DETERMINATION OF METABOLIZABLE ENERGY

	Fresh weight.	Dry matter.	Heat of combustion per gm. of d. m.	Gross energy.	
				Feed.	Excreta.
DAILY FEED (*a*).	*Gm.*	*Gm.*	*Cal.*	*Cal.*	*Cal.*
Prairie hay	1,816.0	1,659.6	4.387	7,280	
Beet pulp	1,816.0	1,672.5	4.075	6,815	
Molasses	908.0	679.8	3.582	2,435	
Blood flour	544.8	495.2	5.739	2,841	
Corn and oats mixed	3,178.0	2,871.6	4.541	13,039	
Total				32,410	
DAILY EXCRETA.					
Cow E-7 (control):					
Feces	10,786	2,241	4.514		10,116
Urine	7,063		.139		981
Methane		196	13.344		2,615
Total					13,712
METABOLIZABLE ENERGY BY DIFFERENCE				18,698	
Cow E-8 (low-P):					
Feces	10,009	2,062	4.681		9,652
Urine	8,202		.116		951
Methane		199	13.344		2,655
Total					13,258
METABOLIZABLE ENERGY BY DIFFERENCE				19,152	
Cow E-9 (low-P):					
Feces	12,522	2,328	4.674		10,881
Urine	9,810		.087		853
Methane		190	13.344		2,535
Total					14,269
METABOLIZABLE ENERGY BY DIFFERENCE				18,141	
AVERAGE, LOW-PHOSPHORUS COWS—METABOLIZABLE ENERGY				18,646	

(*a*) All three experimental cows were on same feed intake during digestion trial.

DISCUSSION OF METABOLIZABLE ENERGY DATA

From the data given in Table XII it will be observed that the loss of chemical energy in the feces of the experimental animals constitutes the greatest percentage of the total loss. This represented 73 per cent of the total energy loss for the control E-7, and 72 and 76 per cent, respectively, for the low-phosphorus cows, E-8 and E-9, or an average of 74 per cent for the deficient animals. This agrees closely with the figure noted for the control and, in general, confirms the results of the digestion trial with these cows, which have been discussed elsewhere.

On the other hand, it will be noted that the losses of chemical energy in the urine form only a relatively small percentage of the total loss of energy in the visible excreta. Furthermore there is no consistent, significant difference between the normal and phosphorus-deficient condition in this respect. This indicates that there is no abnormal loss of energy in the urine of the low-phosphorus cows resulting from the elimination of partially oxidized materials. If anything a higher outgo of energy is noted in the urine of the control, being approximately 9 per cent higher than the average for the two deficient cows. It is not known what significance should be attached to these observed differences.

In total losses of energy, the average for the deficient cows approximates closely the value observed for the control, indicating that a higher outgo of energy through one avenue of excretion has been counter-balanced by a lessened output through another, and that the losses of energy in the visible excreta are in no wise heavier in the phosphorus-deficient than in the normal animal.

It will be observed that on the same feed intake the average metabolizable energy determined for the deficient animals was practically identical with that of the control. This would indicate that the lowered efficiency in the utilization of feed by the low-phosphorus cows must be sought in an increased metabolism rather than through any abnormal losses of energy in the excreta. In order to secure further information in regard to the energy metabolism of animals in the phosphorus-deficient condition, it was decided to make oxygen-consumption determinations, by means of a portable metabolism apparatus, on suitable experimental animals.

PHASE III

ENERGY-METABOLISM STUDIES

Since the digestion trials showed clearly that the phosphorus-deficient animals were assimilating the nutrients of the ration as efficiently as the control, and, likewise, since no greater losses of energy were demonstrated in the visible excreta, it must be concluded that the lowered efficiency of feed utilization in the low-phosphorus condition is probably due to a higher basal metabolism of the animals concerned. Such a metabolism, it is expected, would be made evident in a higher oxygen consumption and correspondingly greater expiration of carbon dioxide than in the normal condition.

The methods of determination, in the past, have been complicated and expensive. While the accuracy of measurements with the respiration calorimeter or respiration chamber is highly desirable, no apparatus of this type was available at the Kansas Agricultural Experiment Station, and since it was not practicable to ship the phosphorus-deficient animals to a station where such measurements could be made, it was necessary to resort to some other means of measuring their energy metabolism.

In recent years Brody and coworkers *(8)* have developed a technique which has involved the direct measurement of oxygen consumption by means of the Benedict type of portable metabolism apparatus, adapted for use with farm animals. The respiratory system of the animal is connected with an oxygen spirometer by means of a rubber sleeve pulled over the animal's muzzle to serve as a mask. Benedict *(5)*, after many years of exhaustive research in perfecting this type of machine, has stated, "The oxygen consumption of patients may be studied by this apparatus with an accuracy fully equal to other standard methods of studying the respiratory exchange." A detailed description of the apparatus for use with farm animals and the results obtained have been reported in Missouri Research Bulletin 143 and subsequent publications *(9, 10)*.

Since this type of apparatus has proved entirely satisfactory for studying the energy metabolism of dairy animals, it was decided to make use of it in the present investigation. Through the courtesy of the Department of Dairy Husbandry of the University of Missouri a portable respiration machine was secured.

ANIMALS USED

Control cow, E-7 (receiving phosphorus supplement): Senior four-year-old, weight 977 pounds, freshened December 24,1930, and producing approximately 40 pounds of milk per day. This animal was not used in the actual oxygen-consumption determinations, but she was used as a control, receiving phosphorus supplement, in all blood analyses and in general reaction to environmental conditions throughout this phase of the investigation.

Two other cows receiving the low-phosphorus ration without supplement were used in these tests: E-13, senior two-year-old; weight 935 pounds, freshened December 15, 1931, and producing approximately 40 pounds of milk per day at the outset of the experiment, (Fig. 3.) E-16, senior two-year-old, weight 954 pounds, freshened January 14, 1932, and producing approximately 40 pounds of milk per day. (Fig. 4.)

While E-7 is designated as the control animal, it was planned to have the low-phosphorus cows serve as their own controls in the oxygen-consumption determinations. That is to say, following the collection of satisfactory energy-metabolism records in the phosphorus-deficient condition, these same animals, E-13 and E-16, would be fed the phosphorus supplement and similar records obtained in the supplemented condition. In this manner it was certain that the individuality of the animals concerned was not a factor, and the results obtained would more accurately represent the influence of level of phosphorus intake.

Feeding and Management of Animals.—In general the feeding of the experimental animals during this phase of the investigation was similar to that in the earlier work. The same feeds were employed throughout, and the ration adjusted at monthly controls, or oftener, as the conditions of the experiment demanded. For the most part the plane of feeding was approximately 110 per cent of the estimated digestible nutrients required, as prescribed by the Morrison feeding standard.

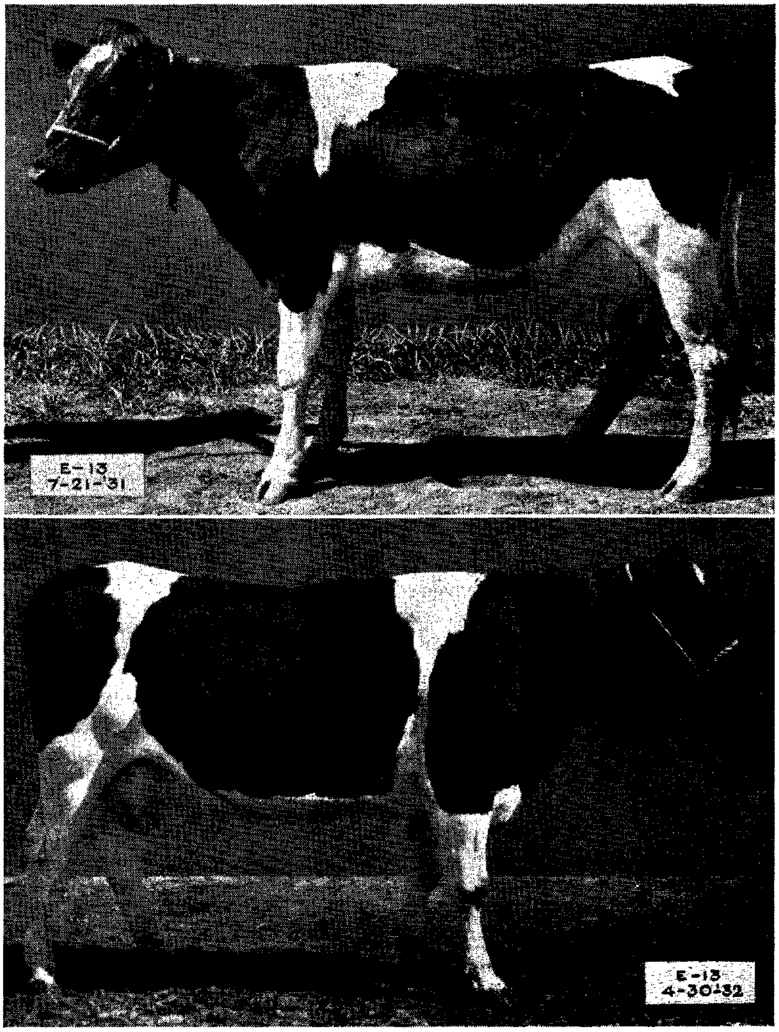

FIG. 3.—Upper—Cow E-13 after five months on low-phosphorus experimental ration, during which time she lost 181 pounds. Lower—The same cow after receiving phosphorus supplement for seven months, during which time she gained 305 pounds.

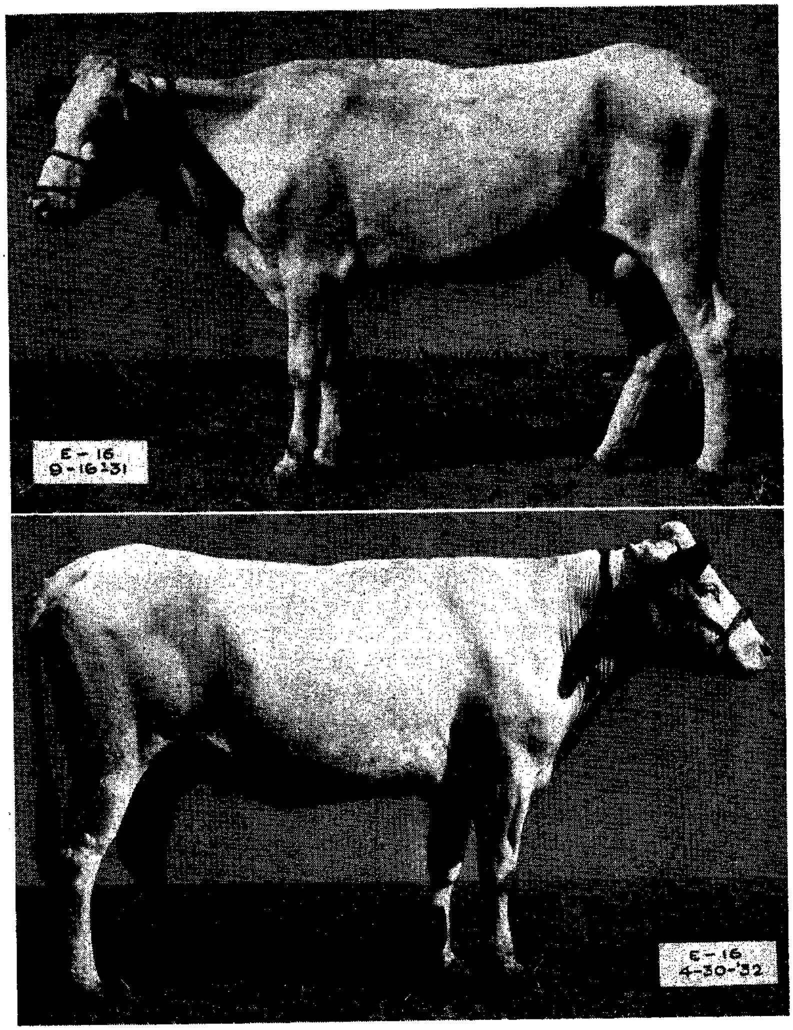

FIG. 4.—Upper—Cow E-16 after six months on low-phosphorus experimental ration, during which time she lost 120 pounds. Lower—the same cow after receiving phosphorus supplement for seven months, during which time she gained 252 pounds.

During the last eight months of the experiment, when a majority of the energy-metabolism data were secured, the ration was comprised as follows: Alfalfa hay, 2 pounds; prairie hay, 6 pounds; beet pulp (dry), 5 pounds; molasses (cane), 2 pounds; blood flour, 1 pound; corn (yellow), 5 pounds; and oats, 2 pounds.

Other details of feeding and management were essentially the same as outlined in the plan covering earlier phases of the problem.

Training of Experimental Animals.–In making metabolism measurements it is essential that the animals be as completely relaxed and as quiet as possible. The Missouri workers have reported best results where the training was started with young animals. There were some objections to starting the work with calves in the present investigation, and, accordingly, it was decided to see what results could be secured employing older animals.

The training of the experimental animals was started the latter part of March, 1931. Proceeding on the assumption that "it is difficult to teach an old dog new tricks," it was anticipated that some little time would be required in accustoming the cows to lying down and remaining quiet while the oxygen-consumption determinations were being made. This was fully borne out in the period of training. It was found necessary to throw the animals every other day for almost three months before they would respond to the commands and remain sufficiently quiet for making the measurements.

It was observed that as these cows became more deficient in phosphorus they were more easily handled, and it required less patience to obtain suitable records than when the animals were on the supplemented ration.

TECHNIQUE OF OXYGEN-CONSUMPTION MEASUREMENTS

All oxygen-consumption measurements were made in the experimental barn in which the cows were regularly stabled. A special stall was constructed with wooden stanchion, in which the animal could lie in a natural position. The stall was always well bedded, and the animal experienced a minimum of discomfort.

All measurements were made approximately 12 hours after the previous evening feeding and just preceding the morning feeding. In this manner the energy metabolism of the animal was determined under "resting conditions." The measurements obtained did not represent the basal metabolism of the animals involved, since this was possible only in the post-absorptive condition. In humans this condition is obtained 12 to 15 hours after moderate feeding, during complete rest. With cattle, however, there is some question as to when the post-absorptive condition is reached, the present view being that it is some time between 30 and 70 hours after feeding. In the present investigation, where it was desired to collect frequent records, it was obviously impracticable to submit the experimental animals to frequent fasts for the length of time noted. Accordingly the practice adopted by Brody and coworkers (8) was followed of making the measurements just before the morning feeding.

Since the morning feeding in the experimental barn was done at 7 o'clock, the cow whose record was to be taken was put down at approximately 5:30, and after a half-hour interval, when it was certain that she was completely relaxed and at rest, the mask was adjusted and at least three records, each of six minutes duration, were run whenever possible. The mask was removed following the completion of each record.

It was found necessary to discard a considerable number of records, representing quite a few mornings' work, because of restlessness on the part of the cows at one time or another. Any movement of the head or shifting of the legs was reflected in the record recorded graphically by the Collins clock-kymograph device. An-

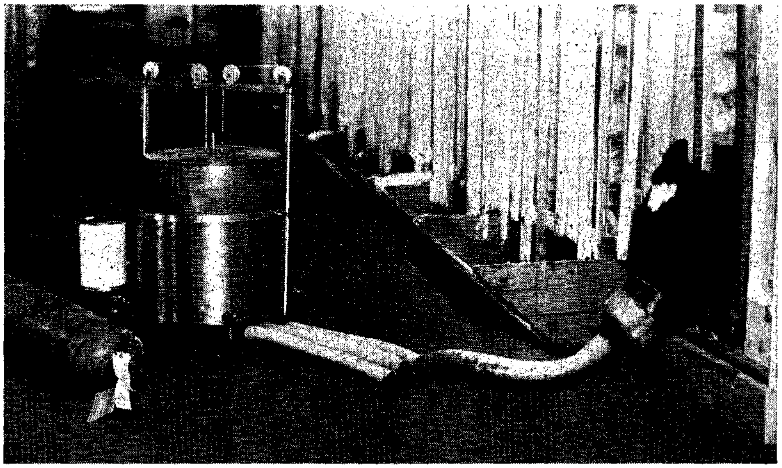

FIG. 5.—Method of measuring oxygen consumption of experimental animals.

other undesirable habit that the cows indulged in frequently was that of chewing the cud with the mask in position. While this indicated a degree of relaxation and comfort, which the technique of the experiment called for, it frequently meant the loss of a record. Notwithstanding the difficulties encountered, a sufficient number of measurements were made to furnish a fairly satisfactory picture of the energy metabolism of the experimental cows in the low-phosphorus and supplemented condition.

In connection with each morning's measurements the practice was followed of taking three temperatures (barn, spirometer, and cow), also the barometric pressure and the weight of the animal. Frequent tests for any leaks in the apparatus were made according to the standard Benedict pocedure.

Figure 5 shows the apparatus set up and connected to the respiratory system of the animal. As the oxygen is used up in the spirometer, the bell descends in its water seal, the course of descent being recorded graphically on the clock-kymograph.

TABLE XIII.—RECORD OF LIVE WEIGHT, FEED CONSUMPTION, AND MILK PRODUCTION OF EXPERIMENTAL ANIMALS THROUGHOUT THE PERIOD OF ENERGY-METABOLISM MEASUREMENTS.

Cow.	Month.	Live weight.	Average daily milk yield.	Approximate digestible nutrients.	
				Consumed.	Refused.
		Lbs.	Lbs.	Lbs.	Lbs.
E-7 (control)	**1931**				
	March	988	43	652	0
	April	1,009	41	677	0
	May	1,029	41	699	0
	June	1,040	38	677	0
	July	1,063	33	546	65
	Aug.	1,048	33	571	0
	Sept. (a)	1,053	22	432	0
	Oct.	1,072	18	516	0
	Nov.	1,129	6	460	0
	Dec.	1,199	Dry	476	0
	1932				
	Jan.	(b) 1,261	....	....	....
E-13 (low-P)	**1931**				
	March	961	37	540	23
	April	903	25	466	107
	May	822	19	361	140
	June	786	16	324	43
	July	819	15	(c) 330	25
	Aug.	821	18	(d) 452	0
	Sept. (a)	864	14	(e) 379	0
	Oct.	884	16	452	0
	Nov.	931	15	460	0
	Dec.	(a) 983	15	399	0
	1932				
	Jan.	976	16	476	0
	Feb.	1,008	15	445	0
	March	1,057	11	476	0
	April	1,096	5	460	0
E-16 (low-P)	**1931**				
	March	944	42	545	17
	April	939	41	619	4
	May	931	36	520	101
	June	856	30	426	48
	July	825	24	(c) 307	48
	Aug.	773	20	(d) 395	56
	Sept. (a)	792	18	(e) 379	0
	Oct.	778	22	452	0
	Nov.	838	22	460	0
	Dec.	(a) 875	13	384	0
	1932				
	Jan.	858	20	476	0
	Feb.	900	18	445	0
	March	952	7	476	0
	April	1,014	Dry	460	0

(a) Three cows on 72-hour fast this month.
(b) E-7 was taken off experiment January 10, 1932.
(c) Feed intake reduced to 100 per cent level for E-13 and E-16; E-13 was fed phosphorus supplement July 21 to August 20.
(d) Feed intake raised to 110 per cent level again for E-13 and E-16; E-16 was fed phosphorus supplement August 26 to September 4.
(e) E-13 and E-16 on phosphorus supplement September 19 to end of experiment.

Before entering into a discussion of the results obtained in the energy-metabolism studies it would be desirable to comment on the general reaction of the experimental cows on the low-phosphorus ration throughout this period. This will be helpful to a proper interpretation of the data obtained in the oxygen-consumption measurements. Therefore a record of the feed consumption, live weight, and milk production is given in Table XIII, with a discussion in detail of the observations made throughout this period.

OBSERVATIONS ON EXPERIMENTAL ANIMALS DURING PERIOD OF THE ENERGY-METABOLISM STUDIES

While E-7, the control, had been receiving the low-phosphorus ration with added supplement (100 grams monobasic sodium phosphate) daily since the completion of previous phases of the investigation, E-13 and E-16 were started on this ration early in March, 1931. These cows had been removed from a long-time experiment on a restricted diet for use in the present experiment. One feature of this diet was that it was below average in phosphorus content, and the phosphorus reserves of these animals were undoubtedly at a low ebb. This fact was borne out in the blood analyses and the early incidence of anorexia in these cows.

Loss of Appetite.—The anorexia noted in earlier work was especially marked in the low-phosphorus cows in this experiment. This apparently was not due to any lack of palatability in the ration, since the data in Table XI show that E-7 was not similarly affected. With the exception of the month of July, when this cow went off feed as a result of sugar injection, no refused feed was recorded for her at any time throughout the experiment. On the other hand E-13 and E-16 showed evidence of failing appetite after receiving the experimental ration for less than a month. This condition was most pronounced in the case of E-13, and during the months of April and May considerable quantities of feed were weighed back for this cow, amounting to approximately 18 and 27 per cent, respectively, of the nutrients furnished.

With E-16, on the other hand, while depressed appetite was evident in March, there was very little feed weighed back for her during April. In May, however, loss of appetite recurred, and approximately 16 per cent of the nutrients were refused in that month. During June and July, anorexia was evident in both cows but in a lessened degree. In the latter month the feed intake was decreased to the 100 per cent level, to reduce the waste of feed as much as possible.

By frequent changes in the ration attempts were made to appeal to the appetites of these cows during the periods of depressed appetite, but with indifferent success. Alfalfa was substituted for a large part of the prairie hay, while the grain fed was increased at the expense of the beet pulp, but these measures served only as temporary palliatives. It was not until the phosphorus supplement was added,to the ration that any substantial recovery in appetite was observed. The supplement was first added to the ration of E-13 July 21 and continued until August 20, while E-16 was fed the supplement for a short period, August 26 to September 4, inclusive. Both cows were finally put on the supplemented ration, starting

September 19, and received it throughout the remainder of the experiment.

As in previous work the marked effect of the added phosphorus was readily observed. Time and again throughout the different phases of the investigation it has been noted that the simple addition of the phosphorus supplement to the ration of an animal which has been refusing appreciable quantities of its feed usually resulted in complete consumption of the ration. And the response in the whole animal organism was almost equally marked. The rapidity of the improvement on the increased phosphorus intake, with animals that have shown marked symptoms of aphosphorosis, was in some respects analagous to the recovery noted in some types of avitaminosis with laboratory animals, when the missing factor was supplied.

Depraved Appetite.—Symptoms of depraved appetite were evident at an early stage in the present work and confirm the previously expressed belief that E-13 and E-16 were already in a condition of low-phosphorus reserves when placed on experiment. Both cows developed the habit of eating shavings, with which their stalls were bedded, in the first month of the experiment. Another habit started about the same time was that of gnawing on their stall chains. Since the control and other animals on experiment in the same barn did not display similar symptoms, it was assumed that these were early indications of phosphorus deficit. About the middle of April E-13 commenced eating dirt and chewing on rocks in the exercise lot. These grosser symptoms of depraved appetite were not observed in E-16, however, until the latter part of May, or after approximately three months on the experimental ration. Once started, these symptoms became more pronounced in degree with both cows, until the phosphorus supplement was added to the ration. While the symptoms of depraved appetite were gradually allayed with the phosphorus feeding, there was still some evidence of abnormal appetite present in both cows nearly three months after the phosphorus feeding was started, indicating, in a striking manner, the extent of their aphosphorosis on the deficient ration.

While E-16 showed possible evidence of greater phosphorus reserve and better adaptability to the phosphorus-deficient ration, in that a longer period was involved before her appetite, live weight, and milk production were affected, on the other hand she showed the more pronounced symptoms of the disorder when these finally became evident. Her case was very similar to that of E-9 in the earlier phases of the investigation. Both cows showed more resistance to the inroads of the deficiency in the early stages of the experiment, but finally displayed the grosser symptoms in more acute form. E-16, like E-9 eventually became quite listless, was given to lying down considerably, and developed a lameness in her right front leg, which responded only in the subsequent period of phosphorus feeding.

Live Weight.—During the period, March 6 to July 1, inclusive, E-13 and E-16 lost 142 and 119 pounds in weight, respectively. This is an average of more than a pound a day for the period involved. During the same time E-7, the control, gained 75 pounds, or an average daily gain of approximately 0.6 pound. While it is apparent that the lowered food consumption of the low-phosphorus cows, due to loss of appetite, is partly responsible for these considerable losses in weight, it does not account for the entire loss. Nor does the increased consumption of nutrients, resulting from the improved appetite following the addition of phosphorus supplement to the ration, wholly explain the substantial increases recorded in live weight for these cows. It is apparent that there is a greater efficiency in the utilization of feed in the phosphorus-fed animal.

Blood Analysis.—The blood analyses, presented in Table XIV, show the inorganic phosphorus content to have dropped to less than 3 milligrams before the end of the first month on the experimental ration, and in E-16 it had decreased to the low value of 2.27 milligrams. It will be observed that this decrease continued until, after approximately three months, values of 1.72 and 1.28 milligrams per 100 c.c. of whole blood were recorded for E-13 and E-16, respectively. Since these cows were showing evident symptoms of abnormal appetite at this time, there was little question about their being in a condition of aphosphorosis, which became progressively severe as the experimental period advanced.

It is interesting to observe the manner in which the blood inorganic phosphorus of the deficient animal reacts to an increased supply of readily available phosphorus in the ration. This is well illustrated in the data given in Table XIV. Within approximately a week's time after the supplement was added to the ration of E-13 and E-16 in the first instance, the inorganic phosphorus concentration of the blood had increased three-fold, and judging from later results with additions of the supplement, the lower the original level of phosphorus in the blood, the more rapid and pronounced is the increase.

Body Temperature, Pulse Rate, and Respiration.—A limited number of observations were made on the body temperature and pulse rate of the experimental animals, which are presented in Table XV. These were started with the deficient cows 10 days after phosphorus feeding was resumed in September. In spite of the short period of phosphorus feeding before the observations were commenced, it was certain the cows, E-13 and E-16, were still very much in the deficient condition. This fact was borne out by the marked increase in pulse rate of the deficient animals as compared with the control. This difference was gradually lessened as the period of phosphorus feeding advanced, until approximately one and one-half months later the pulse rate of these cows had apparently returned to normal.

No significant differences were observed in the body temperatures of the deficient animals and the control.

TABLE XIV.—INORGANIC PHOSPHORUS DETERMINATIONS ON WHOLE BLOOD OF EXPERIMENTAL ANIMALS

Mg. per 100 c. c. whole blood.

Cow	E-7 (control).	E-13 (low-P).	E-16 (low-P).
March	5.10	2.76	2.27
April	4.32	1.31	1.71
May	4.76	1.72	1.28
July	7.62	2.84	1.50
August 1	7.14	(*a*) 7.96	2.57
September 4	5.06	2.73	(*b*) 8.08
September 12	5.33	3.39	3.94
September 28	5.55	(*c*) 6.20	(*c*) 6.84
November	5.92	10.80	7.14

(*a*) Phosphorus supplement since July 21.
(*b*) Phosphorus supplement since August 26.
(*c*) Phosphorus supplement since September 19.

Typical oxygen-consumption charts which were used in the calculations are shown for the experimental animals in both the low-phosphorous and phosphorus-supplemented periods in figure 6. The regularity of these charts reflects the quiet condition of the animals during the time the records were being made. It is of interest to observe the difference in the character of the respiration during the contrasting periods. During the period of low-phosphorus feeding there was a tendency for the most part to a more rapid, shallower breathing than in the supplemented condition, depending on the severity of the disorder.

TABLE XV.—BODY TEMPERATURE AND PULSE RATE

Cow	Body temperature.			Pulse rate.		
	Control.	E-13.	E-16.	Control.	E-13.	E-16.
9–28–'31	102.3	102.0	102.4	48	84	72
9–30–'31	102.0	102.5	102.8	48	76	92
10– 1–'31	102.8	102.0	102.6	52	64	84
10– 8–'31	102.1	101.8	101.9	50	56	58
10–16–'31	101.6	101.5	101.0	64	72	76
11– 5–'31	103.0	101.0	102.0	64	68	64
12– 5–'31	103.0	102.0	101.8	(*a*) 82	64	68

(*a*) High pulse rate due to excitement of animal.

Reproduction of Experimental Animals.—The reproduction of the cows in this phase of the investigation presented a picture similar in many respects to that furnished by the animals used in the digestion phase. There was sufficient evidence of the influence of phosphorus deficit on the rhythm of the œstrus cycle.

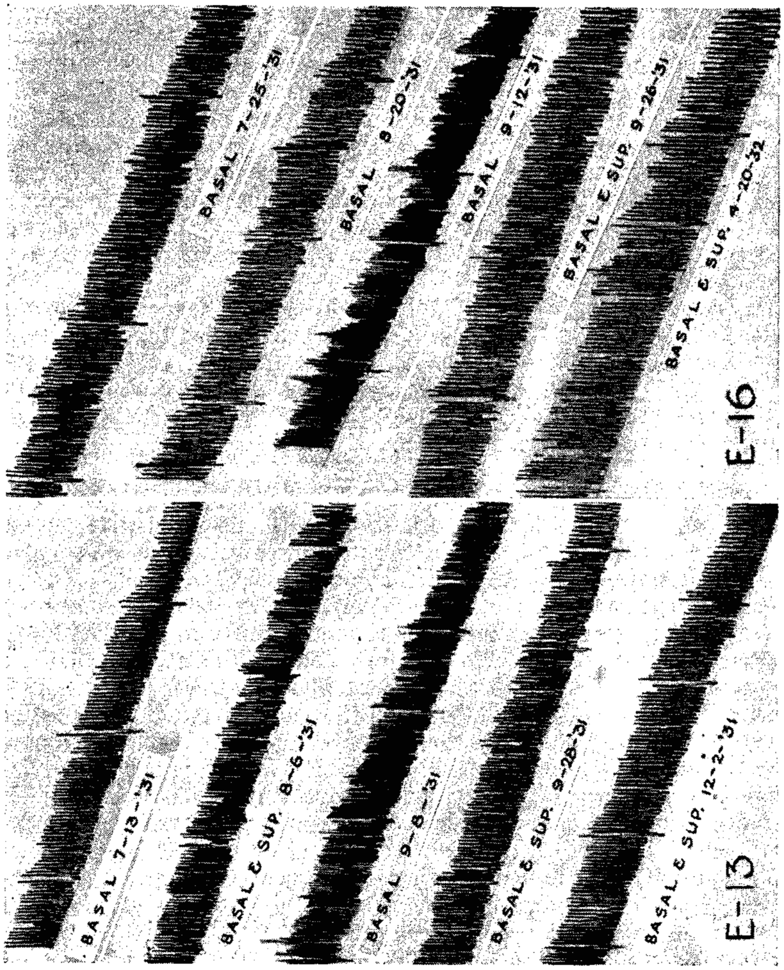

FIG. 6.—Graphic records of oxygen consumption for experimental cows E-13 and E-16 in low-phosphorus and supplemented condition.

The control, E-7, following a normal calving (December 24, 1930), after the digestion phase of the experiment had been completed, displayed regular ovulation periods until bred April 6, 1931. She calved normally the following January.

Although E-13 was started on the low-phosphorus ration early in March, she showed œstrum at regular intervals until May 18, 1931. Following this there was no manifestation of heat in this cow until August 4, which followed a period of phosphorus feeding as indicated in Table XIII. With the increased intake of phosphorus œstrum was observed at regular intervals until she was bred November 25, 1931. She calved normally the following September.

The effects of aphosphorosis as manifested in the inhibition of the œstrum were more pronounced in E-16. It was observed, also, that this cow showed more marked symptoms of phosphorus shortage throughout the periods of low-phosphorus feeding. Started on the phosphorus-deficient ration at the same time as E-13, only one period of œstrum (May 1, 1931) as observed in E-16 before the following October, when she was bred, calving normally in July of the following year. As will be noted in Table XIII, she had been receiving phosphorus supplement for over a month at the time of breeding.

In considering the reproductive behavior of these two cows it, seems evident that E-13, with her more pronounced lapses of appetite and more rapid decrease in milk production, as indicated in Table XIII, did not experience so heavy draughts on her phosphorus reserves, with a consequence that ovulation was not impaired to the extent that it was in E-16.

CALCULATIONS BASED ON OXYGEN-CONSUMPTION CHARTS

The oxygen-consumption method is sufficiently sensitive to any muscular action on the part of the animal that considerable variation in results is to be expected. The data reported represent morning measurements when the cows appeared to be most quiet and relaxed. The record retained was the most uniform record of lowest oxygen consumption. This was justified on the ground that any restlessness or movement on the part of the subject would tend to increase the rate of metabolism. In this manner the curve of lowest oxygen consumption would more nearly represent the true value of the resting metabolism of the animal. By following this method of organizing the data throughout these studies, it is felt that the results should indicate the relative values of the animal's metabolism during the periods of low-phosphorus and supplemented feeding.

The usual method of calculating the oxygen-consumption measurements based on the graph was used. This consists in measuring the rise of the curve for a six-minute period. This rise of the "oxygen-consumption line" is the measure of the fall of the spirometer bell for the same length of time. The bell has a standard capacity of 165.84 ml. per mm. of height. This amount of oxygen (at the average calorific value of 4.825 cal. per liter) equals 0.8 calorie. Therefore, 1 mm. in six minutes represents 8 calories per hour, subject to the standard corrections for temperature, pressure, and moisture.

The numerical data obtained from the calculations based on the oxygen consumption measurements are presented in Table XVI, and graphically in figure 7.

TABLE XVI.—ENERGY-METABOLISM DATA

DATE.	RATION.	Weight (kg.).	Area (a) (sq. m.).	Heat production, cal. for 24 hours. Total.	Per sq. m.	Per kg.	Barn temp. Deg. C.
			E-13				
5-29-'31....	Basal..............	349	3.98	11,346	2,851	32.6	20.0
7-13-'31....	Basal..............	340	3.92	10,535	2,688	30.9	23.0
7-21-'31....	Basal..............	353	4.01	11,140	2,778	31.5	23.5
7-29-'31....	Basal+sup.........	360	4.05	10,351	2,556	28.8	27.0
8- 1-'31....	Basal+sup.........	361	4.05	10,696	2,641	29.6	25.0
8- 6-'31....	Basal+sup.........	359	4.05	10,284	2,539	28.6	25.0
9- 8-'31....	Basal..............	384	4.20	11,287	2,687	29.4	27.0
9-10-'31....	Basal..............	386	4.21	13,328	3,166	34.5	25.5
9-12-'31....	Basal..............	355	4.02	13,802	3,433	38.8	25.0
9-28-'31....	Basal+sup.........	391	4.24	10,541	2,486	27.0	17.0
11-30-'31....	Basal+sup.........	432	4.48	12,151	2,712	28.1	10.0
12- 2-'31....	Basal+sup.........	426	4.45	12,909	2,901	30.3	8.0
3- 1-'32....	Basal+sup.........	470	4.70	12,742	2,711	27.1	16.0
4-19-'32....	Basal+sup.........	509	4.92	12,572	2,555	24.7	15.0
Average of six low-P determinations..............				11,906	2,934	32.9	....
Average of eight supplemented determinations...				11,531	2,637	28.0	....
9-15-'31....	Basal (after fasting 72 hrs.)..........	368	4.10	8,912	2,174	24.2	27.5
12-11-'31....	Basal+sup. (after fasting 72 hrs.)....	423	4.43	10,067	2,272	23.8	12.0
			E-16				
5-29-'31....	Basal..............	383	4.19	12,586	3,004	32.9	21.0
7-18-'31....	Basal..............	350	3.98	11,544	2,901	32.9	25.0
7-25-'31....	Basal..............	343	3.94	11,351	2,881	33.1	24.5
8-20-'31....	Basal..............	330	3.85	12,994	3,375	38.6	21.0
9- 9-'31....	Basal..............	347	3.97	11,710	2,950	33.7	26.0
9-12-'31....	Basal..............	337	3.90	14,416	3,696	42.8	24.5
9-26-'31....	Basal+sup.........	340	3.92	11,182	2,853	32.9	14.5
11-22-'31....	Basal+sup.........	381	4.16	12,257	2,932	32.2	13.0
2-27-'32....	Basal+sup.........	420	4.42	12,538	2,876	30.0	14.0
4-20-'32....	Basal+sup.........	470	4.70	12,472	2,654	26.5	15.0
Average of six low-P determinations..............				12,434	3,135	35.7	....
Average of four supplemented determinations......				12,156	2,829	30.5	....
9-15-'31....	Basal (after fasting 72 hrs.)..........	327	3.84	10,941	2,849	33.5	27.5
12-12-'31....	Basal+sup. (after fasting 72 hrs.)....	373	4.13	11,016	2,667	29.5	10.0
			E-13 + E-16				
Average of 12 low-P determinations..............				12,169	3,034	34.3	....
Average of 12 supplemented determinations.......				11,739	2,701	28.8	....

(a) The surface area was computed according to the formula SA = 0.15 W.56 (Mo. Research Bul. 89), in which SA is the surface area in square meters and W the weight in kilograms.

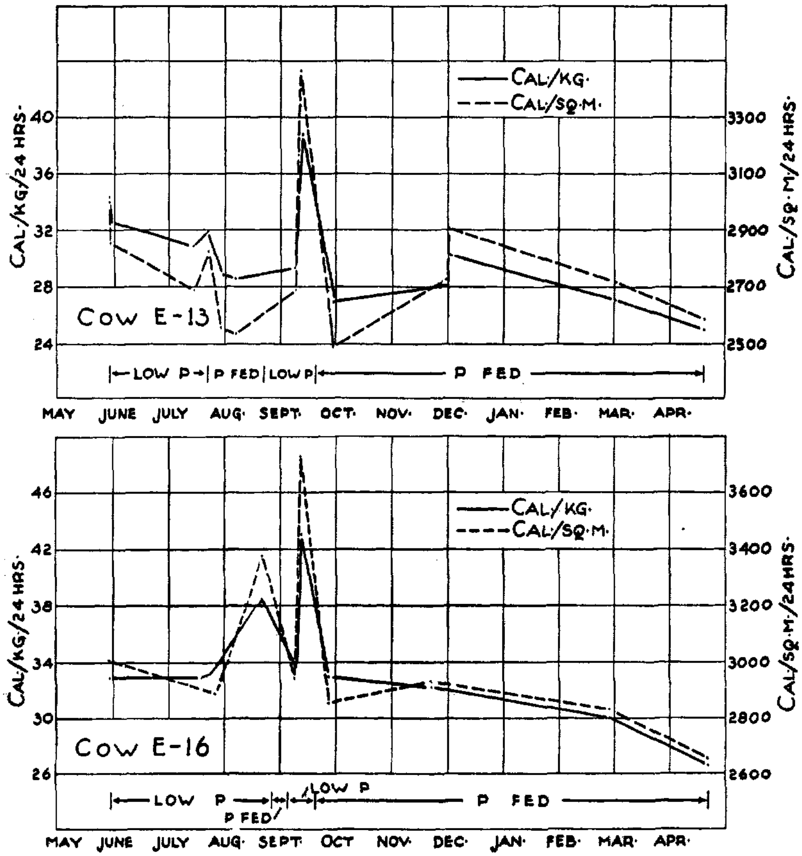

FIG. 7.—Graphic presentation of energy-metabolism data for cows E-13 and E-16.

DISCUSSION OF ENERGY-METABOLISM DATA

The training of the cows for the oxygen-consumption measurements was started the latter part of March, 1931, and approximately three months of intensive training were required before satisfactory records could be secured. The first measurements which were deemed satisfactory were made the latter part of May. The collection of data was continued throughout the following 10-month period.

In considering the data presented in Table XVI, and graphically in figure 7, it should be emphasized that while the experimental animals were fed about the 110 per cent level of nutrient intake, loss of appetite was a factor to be contended with early in the period of low-phosphorus feeding. This fact has been brought out clearly in the discussion of the data given in Table XIII. With one or two exceptions already noted, it was only in the last nine months of the experiment (August, 1931, to April, 1932) that E-13 and E-16 were consuming all of the feed furnished. This fact should be borne in mind in interpreting the results of the oxygen-consumption measurements. The metabolic stimulus of food is well recognized and has been commented on by many workers in this field. It is reasonable, therefore, to expect that the metabolism of these cows would have been higher than the results obtained, had they been consuming the full amount of the ration offered in the early months of the experiment.

Throughout the period in which these determinations were made there was a considerable decrease in the lactation of the experimental cows. Since Brody and coworkers (9) have found that lactating dairy cows show a metabolic level 30 to 60 per cent above the general level of metabolism, it might be inferred that part of the decrease in oxygen consumption during the later stages of the experiment could be accounted for by the lowered intensity of lactation. Brody, however, suggests that "the causative factor for the high metabolism during lactation may not be the process of lactation as such, but chiefly the heat increment of the extra food consumption during lactation."

In the present investigation, as noted in Table XIII, the feed consumption was not reduced as the milk flow decreased, but was materially increased on each occasion that phosphorus was added to the ration because of the improved appetite of the cows. On this basis the change in lactation level would not account for the decrease in metabolism observed in both cows toward the end of the experiment.

It is apparent from a study of these data that there is a general tendency for higher oxygen consumption, indicating a higher metabolism, on the part of the animals in the phosphorus-deficient condition. With the addition of phosphorus supplement to the ration, the metabolism tends to decline.

It will be noted that short periods of phosphorus feeding are indicated for E-13 and E-16, starting the latter part of July and August for each cow, respectively. This was done because of the general weakened condition of these animals and also to stimulate their appetites. After partial recovery the phosphorus supplement was withdrawn. A significant drop in the rate of metabolism is indicated during this short period of phosphorus feeding. Furthermore, when the supplement was withdrawn from the ration, there was a marked rise in the energy metabolism of both cows. On September 19 the two animals were again placed on the supplemented ration, which they received until the end of the experiment. As in the previous case, this was followed by a marked decline in oxygen consumption for both cows, indicating that the addition of phosphorus was in some way responsible for a lowering of the general metabolism of the individual.

Average values for the metabolism of each cow in terms of total heat production for 24 hours, and in relation to body weight and surface area, have been given in Table XVI. It will be noted that the energy metabolism for E-16 was on a higher level in both the low-phosphorus and supplemented conditions than for E-13. This is probably explained in part by the better appetite of E-16, resulting in a higher food consumption, especially in the periods of low-phosphorus feeding. On the supplemented ration this difference was not so marked.

It will be observed in Table XVI that oxygen-consumption determinations were made on both animals in the low-phosphorus and supplemented condition at the end of a fasting period of 72 hours duration. As it was not practicable to repeat the fasting condition in these animals, the observations were limited to four determinations at the conclusion of each fasting period with the results noted. While these data are too limited to warrant definite conclusions, it is interesting to note that one of the cows, E-16, showed a distinctly higher basal metabolism during the period of low-phosphorus feeding.

The general summary of the energy-metabolism values obtained during the period of low-phosphorus feeding gave an average figure for the two animals of 3,034 calories per square meter and 34.3 calories per kilogram per 24 hours, while similar determinations on these animals on the supplemented ration gave an average value of 2,701 calories per square meter and 28.8 calories per kilogram.

The higher metabolism of the phosphorus-deficient cows would help to explain the lowered efficiency in feed utilization of animals afflicted with this disorder. It is recognized, however, that the relatively few determinations presented in this paper are not sufficient to establish the magnitude of the effect of phosphorus deficiency on metabolism. For this reason additional data will need to be secured, both as to number of animals and measurements involved, before it can be determined whether the increased metabolism can account for the entire difference in the utilization of feed by the normal and the phosphorus-deficient animal.

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